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Artificial Organs

P1 (EI0184)

ACCUMULATION RATE OF AGEs IN THE SKIN BIOPSY TISSUE OF DIALYSIS PATIENTS

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Objectives: To measure the accumulation of different Advanced glycation endproducts (AGEs) in a period of 1 year in diabetic hemodialysis (HD) patients and to find the factors that influence their accumulation.
Methods: Twenty diabetic HD patients were enrolled in this study. Skin biopsy was performed twice with an interval of one year. High-performance liquid chromatography was performed on the skin biopsies in order to measure their pentosidine, carboxymethyl-lysine (CML) and carboxyethyl-lysine (CEL) content. Skin Autofluorescence (AF) was used as an additional method to estimate AGEs accumulation. Dietary records from the HD patients were obtained to assess the calorie, protein and AGE intake. Body Mass Index (BMI), as a measure of nutritional state, was calculated.
Results: Pentosidine (59.9±43.6 vs. 83.5±59.9; p=0.002), CML (1529±1038 vs. 2050±1204; p=0.012) and CEL (505±387 vs. 715±658; p=0.015) were significantly increased in skin biopsy specimens during the period of one year. Skin AF correlated with pentosidine, CML and CEL from the skin biopsies (R=0.902 p=0.001; R=0.875 p=0.001; R=0.654 p=0.002). Pentosidine, CML and CEL content of the skin biopsies did not correlate with the calorie, protein and AGEs intake. In the multivariate analysis we found that independent predictors of the annual increase of pentosidine were: CRP (p=0.038), the annual increase of Skin AF (p=0.01) and BMI (p=0.041). The annual increase of Skin AF (p=0.021) and BMI (p=0.010) were also the independent predictors of the annual change of CML, whereas the independent predictor of the annual change of CEL was the annual increase of Skin AF (p=0.001).
Conclusions: Skin AGEs increased rapidly in diabetic HD patients. The Skin AF is a good estimate of AGEs accumulation. The BMI and CRP are independent predictors of AGEs accumulation.

P2 (EI0340)

NEPHROLITHIASIS IN AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE

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Objectives: The prevalence of nephrolithiasis is considerably greater in patients with autosomal dominant polycystic kidney disease than in the general population. The anatomic factors because of increased intrarenal obstruction, such as cyst growth, renal tubular stasis and methabolic disorders, are important and may predispose to stone formation.
Methods: In order to evaluate the nephrolithiasis in polycystic kidneys, 60 patients with autosomal dominant polycystic kidney disease, mean age 42.6+/-12.8 years, underwent echosonography and computed tomography scan. Routine blood analysis and urine samples, including 24 h urine collections, were done.
Results: Renal stones were detected in 22 out of 60 patients (36.6%). The morphologic data presented that patients with autosomal dominant polycystic kidney disease and nephrolithiasis had more renal cysts and larger predominant cyst size than patients without nephrolithiasis ($p<0.05$). Renal function expressed by creatinine clearance was also different between the 2 groups of patients (72.6+/-9.4 in patients with nephrolithiasis, and 93.7+/-8.6 in patients without nephrolithiasis). Twenty-four-hour urine analysis showed that patients with nephrolithiasis had significantly lower urine volumes and levels of uric acid. Three patients had urinary tract obstruction, ureterolithiasis with hydronephrosis, with diminished creatinine clearance, but after desobstruction and elimination of the calculi, the renal function was improved.
Conclusions: The authors consider that nephrolithiasis an important factor for the progression of the renal demage in patients with autosomal dominant polycystic kidney disease, because of complications that may accelerate the progression of the renal disease and the chronic renal failure.

P3 (EI0312)

USE OF A RENAL TUBULE CELL LINE (HK-2) TO STUDY THE NEPHROTOXIC POTENTIAL OF DIALYSATE TAKEN FROM HIGH CUT-OFF HEMODIALYSIS TREATMENTS IN PATIENTS WITH LIGHT CHAIN INDUCED MYELOMA KIDNEY

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Objectives: Acute kidney injury is common in patients with multiple myeloma (MM), most frequently caused by cast nephropathy, a direct consequence of the high serum free light chain (FLC) concentrations present in these patients. In this condition FLC induced cell stress responses are frequently seen in proximal tubular cells (PTCs), which result in production of cytokines and tubulo-interstitial inflammation. Removal of circulating FLC with extended high cut-off hemodialysis (HCO-HD) has recently been studied and rapid reduction in serum FLC levels was associated with improved likelihood of kidney function recovery. The aim of this investigation was to study renal epithelial cell toxicity of dialysate obtained from HCO-HD treatments of MM patients.
Methods: Spent dialysate collected from HCO-HD treatment sessions of 13 patients with FLC induced AKI were concentrated by filtration with a 5 kDa cut-off membrane. The concentrates were exposed to HK-2 cells, a proximal tubule epithelial cell line from human kidney, for up to 48 hours. The effects on cell morphology and activation were studied by microscopy and by measurement of cytokines in the supernatant using enzyme-linked immunosorbent assay. FLC concentrations were determined by nephelometry, using a particle-enhanced immunoassay.
Results: Incubation of the FLC containing concentrates (25 µmol and 50 µmol) with HK-2 cells induced the release of interleukins IL-6, IL-8 and monocyte chemoattractant protein-1 (MCP-1) and lead to morphological alterations of the tubular cells. There was a considerable variability among the dialysates obtained from different patients. The amount of FLC that stimulated expression of inflammatory cytokines in the PTCs was well within levels that are seen in patients with MM.
Conclusion: FLCs removed from the circulation of MM patients by HCO-HD induce pro-inflammatory responses in proximal tubule epithelial cells. These responses represent an important mechanism of the tubulo-interstitial inflammation frequently seen in the kidneys of MM patients.

P4 (EI0247)

USE OF RIFLE CLASSIFICATION IN PATIENTS WITH COMMUNITY ACQUIRED ACUTE KIDNEY INJURY (CA-AKI)

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Objectives: Acute kidney injury (AKI) is a syndrome with an uncertain follow-up and often with a fatal outcome. The RIFLE and AKIN initiatives have provided a unifying definition for AKI. Present study aims at validating most recent AKI classification system in CA-AKI.
Methods: We analyzed the clinical outcome in 112 pts with CA-AKI. We excluded dialysis pts, those with malignancy and with pre-existing chronic kidney disease (CKD) or kidney transplantation. RIFLE criteria were implied on admission, with retrospective analysis of previously prospectively collected data.
Results: Pts median age was 45.5y, 61.6% were male. 35.7% had 1, 25.4% had 2 and 7.1% had 4 comorbid diseases. Mortality rate was 22.7% and initial mean APACHE2 score was 17.3±7.4. 7 risk factors were implicated in pts outcome. According to RIFLE, pts were classified in stage 1 (Risk) in 1 (0.9%), stage 2 (Injury) in 4 (3.6%) and stage 3 (Failure) in 76 (67.9%). Mortality rate in stage 3 was 18 (16.82%). After 4w of treatment, we found that 31 (27.7%) where in stage 4 (Lost) with a mortality rate of 7 (6.5%). Univariate analysis of four RF like creatinine(s), age (years), UO and APACHE2 in stage 3 and 4 of RIFLE, in correlation with mortality, were significant only with UO. Pts who died (18 in stage 3 and 7 in stage 4), had lower baseline levels of Creat(s) (p=0.028) and UO (0.017) than those alive at 4w. Higher APACHE2 score was associated with higher mortality. Kaplan-Meyer surviving curve showed that RIFLE stage 3 pts were with longer surviving in period of 4w compared to those in stage 4.
Conclusions: The study supports the use of RIFLE as an optimal classification system to stage CA-AKI severity, still there is perhaps a need for using other new parameters in this type of AKI.

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P5 (EI0246)

RENAL AFFECTION IN PATIENT WITH LATE DIAGNOSED SJöGREN'S SYNDROME

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Objectives: Sjögren's syndrome (SS) is the second most common autoimmune disease affecting mainly women. The true prevalence of SS is unknown but is estimated to affect 1–3% of the adult female population. Both tubular and glomerular damage have been described in SS, although glomerular disease is rare. The aim of this report is to present a case of interstitial nephritis with proteinuria in late diagnosed primary Sjögren's syndrome (pSS), aiming to suggest recommendations for treatment.
Methods: We describe a rare case of primary SS (pSS) in a 76-year-old woman presenting with hypokalaemic cardiac arrhythmia, chronic renal failure due to severe tubular and glomerular affection.
Results: The patient had been diagnosed as having pSS on the basis of dry eyes, dry mouth, weight loss, arthralgia, parotid gland tumefaction, positive SSA and positive Schrimer's test. Clinical presentation at admission was cardiac arrhythmia with acute over chronic renal failure with intermediate range of proteinuria. The patient had more than ten years of hypertension as a medical comorbidity. We performed renal biopsy and found global glomerulosclerosis, with mild tubule interstitial nephritis accompanied with interstitial fibrosis and atherosclerotic changes. Immunohistochemical tissue analysis showed multifocal lymphocytic infiltrate. MDRD at point of renal biopsy was 5.01 mL/min. A treatment with corticosteroids (1mg/kg/day) was started. The patient was set to chronic dialysis program. Few weeks later she broke her hip and femur fracture was confirmed. Bone mineral density revealed osteopenia of the hip and normal density of spine. Vitamin D levels were low, supporting the diagnosis of osteomalacia.
Conclusions: The kidney may be a target of the disease in pSS. Although, overt renal disease is rare, latent involvement has been reported in up to one-third of patients. Further studies and successful cases are required to determine indications for and dosages of immunosuppressive treatment in patients with renal involvement of pSS.

P6 (EI0022)

EGFR IS A POOR PREDICTOR OF UREMIC TOXIN CONCENTRATIONS

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Objectives: The degree of chronic kidney disease (CKD) is currently expressed in terms of Glomerular Filtration Rate (GFR), which can be determined directly or estimated according to different formulae based on serum creatinine (SCrea) and/or cystatin C measurements (eGFR). We aimed to investigate whether eGFR-values are representative for uremic toxin concentrations in patients with different degrees of CKD.
Methods: Associations between eGFR based on serum cystatin C (Stevens) and different uremic solutes (MW range 113-240Da) [SCrea, uric acid (UA), symmetric dimethylarginine (SDMA), asymmetric dimethylarginine (ADMA), and free and total hippuric acid (HA), 3-carboxy-4-methyl-5-propyl-2-furanpropionic acid (CMPF), indoxyl sulfate (IS), indole acetic acid (IAA), and p-cresylsulfate (PCS)] were evaluated in 95 CKD patients not on dialysis. The same analysis was applied for 6 other eGFR formulae.
Results: There was a substantial disparity in fits among solutes. In linear regression, SCrea showed the best model fit (Stevens R^2=0.605), while explained variance of eGFR was extremely low for the majority of solutes with R^2 in the range 0.4-0.2 for total IS, SDMA, free IS, and free IAA, and even below 0.2 for ADMA, free HA, free pCS, total HA, total IAA, UA, and CMPF. The other eGFR formulations gave comparably disappointing results with regard to their association to uremic solute concentrations. Relative similarity in R^2 values per solute for the different eGFR values, and the strong disparity in values between solutes, suggest that the differences in R^2 are mainly due to discrepancies in solute handling apart from GFR.
Conclusions: eGFR is poorly associated with concentrations of all studied uremic toxins in patients with different degrees of CKD, and correlates differently with each individual solute. Hence, eGFR cannot be considered representative for evaluating the accumulation of solutes in the course of CKD.

P7 (EI0071)

ROLE OF KILLER-CELL IMMUNOGLOBULIN-LIKE RECEPTOR AND HUMAN LEUCOCYTE ANTIGEN IN KIDNEY TRANSPLANTATION

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Objectives: Innate immunity represents a new frontier in the field of transplantation. Natural killer (NK) cells in particular have a role as a bridge between the innate and adaptive immunity. Killer-cell immunoglobulin-like receptors (KIRs) belong to a polymorphic family of activating and inhibitory receptors expressed on the surface of NK cells and recognize human leukocyte antigen (HLA) class I ligands. The aim of this study was to investigate if KIR/HLA compatibility affects renal allograft survival on the long term.
Methods: We studied 113 patients who received kidney transplant between 1999 and 2005. Eighty-six kidney transplant recipients had a stable renal function, while 26 showed a decrease of their renal function by 20% 5 years post-transplant. The two groups of patients were matched for sex, donor and recipient age, dialysis vintage, cold ischemia time and therapy. All the patients were typed using HLA and KIR-SSO genotyping test. We analyzed the presence of single KIR genes and haplotypes in relation to the decrease of renal function by 20%. Finally we examined all the possible matches/mismatches between KIR genes and known HLA ligands in donor/recipient pairs.
Results: The presence of the KIR2DS3 gene was associated with a better trend of serum creatinine and MDRD over time ($p<0.05$), while in the presence of the ligand, the serum creatinine and MDRD trend seems to worsen in the long term. The analysis performed according to whether there was deterioration of renal function or not, showed that the absence of the KIR2DL1 gene is strongly associated with an increase of 20% of the creatinine value at 5 years, suggesting a potential protective effect given by this gene.
Conclusions: Our data suggest that KIR genes and their respective HLA class I ligands may influence long-term graft outcome after renal transplantation.

P8 (EI0280)

POSSIBILITY OF QUANTUM ENTANGLEMENT BETWEEN ARTIFICIAL ORGAN, IMMUNE SYSTEM AND PATIENT

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Objectives: Analyze the possibility of a quantum entangled between artificial organ (Ao), immune system (Is) and patient (Px).
Methods: The quantum interaction between artificial organ (Ao), immune system (Is), and patient (Px), can be described with the GHZ quantum formalism by wave function: $\Psi AoIsPx\rangle = 1/\sqrt{2}(|Ao\uparrow Is\uparrow Px\uparrow\rangle + |Ao\downarrow Is\downarrow Px\downarrow\rangle)$, where, by analogy with GHZ quantum model, we could consider two ($\uparrow$ and $\downarrow$) of the maximally entangled states of three quantum entities ΨAo, ΨIs, ΨPx.
Results: The wave function $\Psi AoIsPx\rangle$ is produced by maximum entanglement between the various states of the ΨAo, Ψ Is, Ψ Px. Artificial organs could be in a quantum state of functionality Ao$\uparrow$ or non- functionality Ao$\downarrow$, the immune system could be in a quantum state of non-rejection Is$\uparrow$, or rejection Is$\downarrow$, and the patient could be in a quantum state of wellness Px$\uparrow$, or non-wellness Px$\downarrow$. Now, we could describe this entangled states: $\Psi AoIsPx\rangle = 1/\sqrt{2}(|Ao\uparrow Is\uparrow Px\uparrow\rangle + |Ao\downarrow Is\downarrow Px\downarrow\rangle)$; $\Psi AoIsPx\rangle = 1/\sqrt{2}(|Ao\uparrow Is\uparrow Px\downarrow\rangle + |Ao\downarrow Is\downarrow Px\uparrow\rangle)$; $\Psi AoIsPx\rangle = 1/\sqrt{2}(|Ao\uparrow Is\downarrow Px\uparrow\rangle + |Ao\downarrow Is\uparrow Px\downarrow\rangle)$; $\Psi AoIsPx\rangle = 1/\sqrt{2}(|Ao\uparrow Is\downarrow Px\downarrow\rangle + |Ao\downarrow Is\uparrow Px\uparrow\rangle)$.
Conclusions: In GHZ-type quantum states, the equation $\Psi AoIsPx\rangle = 1/\sqrt{2}(|Ao\uparrow Is\uparrow Px\uparrow\rangle + |Ao\downarrow Is\downarrow Px\downarrow\rangle)$ could be seen as the best therapeutic possibility in the quantum states after implant. Indeed, artificial organ (Ao), immune system (Is) and patient (Px) are entangled as "functionality" for artificial organ, "non-rejection" for immune system, and "wellness" for patient. The maximally entangled GHZ-type states could be used to predict that entanglement between artificial organs, immune system, and patient could be therapeutic, where only one maximally quantum entangled state could be therapeutic.

P9 (EI0043)

POST-TRANSPLANTATION IMMUNOLOGIC MONITORING OF RENAL ALLOGRAFT RECIPIENTS TO DIFFERENTIATE INFECTIONS AND GRAFT REJECTION

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Objectives: The two major complications after any solid organ transplantation

are graft rejection (a result of immune conflict) and infection processes (bacterial, viral, fungal). Each one requires a very specific and absolutely adequate therapeutic approach: immunosuppression or anti-infective agents. This is the reason why we need a very exact differentiation between both.
Methods: Our model of immunologic monitoring (IM) includes several highly informative tests to evaluate the dynamic immunoreactivity of the recipients of renal allografts (108 patients, 64 male, 44 female). The T-helper and T-suppressor cell activity and the index Th/Ts, the macrophage activity NBT-test, the enzymes SDH, alpha-GPDH, LDH and their quantitative levels, the NK- and K-cell activity, the RBT, the monocyte test for Fc and C3 receptors, the warm and cold antibodies, the TNAB morphologic analysis, as well as the level of immunodefficiency, allow us to determine the immune state of the patients in different phases after transplantation and more important, to predict the forth-coming complications.
Results: Thus, the proper and exact differentiatial diagnosis of graft rejection and infections requires the adequate therapy for each case and eliminates the possible mistakes, such as high-dose immunosuppression when no rejection is detected or anti-infective agents when only the immune conflict is demonstrated.
Conclusions: Our model of IM allows a precise evaluation of the immunoreactivity of the recipient of renal allografts in any phase after transplantation and can predict the post-transplantation complications.

P10 (EI0108)
ASSESSMENT OF THE HEMOGLOBIN GLYCATION APPLYING MATHEMATICAL MODELLING AND CULTURING OF THE HUMAN ERYTHROCYTES *IN VITRO*

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Objectives: Glycated hemoglobin A1c (HbA1c) is the most commonly used parameter characterizing the long-term metabolic control either in a routine diabetes therapy or in experimental treatments aiming at the substitution or supplementation of the pancreatic insulin secretion. The objective of the study was to assess the ability of a mathematical model of the hemoglobin glycation to reproduce changes of HbA1c occurring in cultures of the human erythrocytes *in vitro* in response to different glucose concentrations.
Methods: The model that was used assumed that HbA1c formation in each erythrocyte obeyed first order kinetics in respect to hemoglobin and glucose and that glycation reaction was lasting throughout the whole erythrocyte life span *in vivo* and was continuing till its apoptosis *in vitro*. The overall glycation rate and HbA1c in each equal-aged group of erythrocytes was estimated individually for each subject based on the results of continuous glucose monitoring *in vivo*. Three constant glucose concentrations were applied in the culturing media *in vitro* (5.2, 10.5 and 15.7 mmol/L). The cultivation lasted for 4-5 weeks. The study group consisted of 10 non-diabetic volunteers (8 females and 2 males).
Results: The mean difference of HbA1c (MD) predicted by the model and measured *in vitro* was equal to –0.54%±0.62%, –0.30%±0.50% and +0.12%±0.71% (p= 0.011), and the mean absolute difference (MAD) was equal to 0.62%±0.54%, 0.56%±0.39% and 0.66%±0.58% (p= 0.53) for three glucose concentrations tested, respectively. Predictions of the model were significantly more accurate during the first 2 weeks of the erythrocytes cultivation (p= 0.0000002).
Conclusions: The obtained mean values of MD and MAD indicated high ability of the model to reproduce relationship of HbA1c and glucose *in vitro*. The model used *in vitro* was identified based on the *in vivo* glucose data, which confirmed also its applicability under *in vivo* conditions.

P11 (EI0412)
BLOOD PRESSURE, ANTI-HYPERTENSIVE TREATMENT AND GRAFT SURVIVAL IN RENAL TRANSPLANT RECIPIENTS USING ELDERLY LIVING KIDNEY DONORS

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Objectives: Hypertension is common following renal transplantation and adversely affects graft and patients survival. Strategies for anti-hypertensive therapy and target blood pressure have not been yet defined. LRT is still predominant in the Balkan countries. The aim of the study is to investigate the role of hypertension on graft survival among the recipients with older kidney donors.
Methods: We performed 230 LRT in the last 20 years, 90 with donors older than 65 years (ED). The recipients mean age was 45±6. Standard immunosuppression was used. The Kaplan Meier 5-year graft survival and renal function were analyzed and compared with the group of 110 younger donors (mean age 53.4) and their recipients (mean age 32.2-YD). Blood pressure was determined retrospectively from the mean three clinic readings and anti-hypertensive drugs. Patients were stratified as controlled (CBP < 140/85, n= 170) with one or more anti-hypertensive drugs, and uncontrolled (UBP >140/85, n=60). The patients received CCB, ACEI, ABR, BB and diuretics.
Results: The 5-year graft survival rate in the ED was 76% compared with 81% in the YD (ns). The serum creatinine at the end of follow-up was 146.04 in ED compared with 123.38 in YD (p< 0.001). Five years after transplantation 96% of the transplant recipients in ED received at list 1 while 54% 2 or 3 additional anti-hypertensive drugs, compared with 85% and 41% of the YD, respectively. The controlled and uncontrolled hypertensive transplant recipients are equally distributed in both ED (CBP= 69%, UBP = 31%) and YD (CBP=73% and UBP= 27%).
Conclusions: CBP contributes to the satisfied 5-graft survival and renal function in ED. The authors confirmed the beneficial effect of anti-hypertensive drug treatment on graft function and survival. Therefore, the use of elderly living donors remains a valuable source of kidneys.

P12 (EI0028)
ESTABLISHMENT OF RAT HEPATOCYTE PRODUCING HIGH MOBILITY GROUP BOX 1 INHIBITOR

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Objectives: One solution for severe hepatic failure may be a hybrid bioartificial liver device containing functioning hepatocytes (Shinoda M. J Surg Res. 2007). High Mobility Group Box 1 (HMGB1) is known as a key mediator in acute liver faulure (Takano K. Shock. 2010). A domain of HMGB1, the A box, competes with HMGB1 for binding receptors and attenuates HMGB1-induced inflammation. In this study, we established rat hepatocyte producing High Mobility Group Box 1 inhibitor, A box.
Methods: We established three types of adenovirus vectors encoding A box. The vectors included a wild type (type W) and 2 mutant types (type A and type N). We transfected the three types of vectors to primary cultured rat hepatocytes, respectively. The culture supernatant was subjected to western blot analysis for A box protein. The supernatant obtained from the culture with type N was also subjected to an *in vitro* test of TNF release inhibition from macrophage (RAW 264.7); macrophage was cultured with recombinant HMGB1 or both recombinant HMGB1 and the supernatant containing A box protein.
Results: Western blot analysis showed a clear expression of A box protein in the culture supernatant of transfected rat hepatocytes in all types of vectors. The expressions were observed from 2 to 4 days after transfection. The expression was much stronger in type A and type N than in type W. TNF release from macrophage was significantly suppressed in the culture of both HMGB1 and A box compared to that of HMGB1 only [401±22 in A box(-) vs 248±16 in A box (+), pg/mL, p<0.05].
Conclusions: Rat hepatocyte producing HMGB1 A box protein was established. Incorporation of this hepatocyte into a hybrid bioartificial liver is of great interest.

P13 (EI0016)
CHRONIC LIVER DAMAGE CORRECTION BY MEANS OF INTRACORPOREAL BIOARTIFICIAL LIVER UNIT (BLU), CONTAINING LONG-TERM SURVIVING DONOR CELLS

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Objectives: Working out of BLU for a long-time supporting of damaged liver is an actual problem of modern hepatology. This research was undertaken for the creation of intracorporeal BLU, containing long-term functioning liver cells (LC) and multipotent mesenchymal stromal cells (MMSC), attached on biodegradable matrix.
Methods: Hepatic failure was modeled in Wistar rats by using CCl_4 according

to the standard scheme within 6 weeks. Adult healthy Wistar rats were used as donors of MMSCs and LCs. MMSCs were obtained by standard procedure and cultivated during 10 days in DMEM with additions. Isolated LCs were obtained by a standard procedure, with 0.12% collagenase solution. The suspensions of LCs and MMSCs were mixed, seeded on biodegradable matrix *Sphero*®GEL as 2,0-4,0x10^6 cells/cm^3 and co-cultivated within 3 days. Matrixes with attached LCs and MMSCs as BLU were transplantated into damaged livers. Dynamics of hepatic failure reduction, and survival LC were investigated in 30, 60, 90 and 180 days after BLU transplantation.
Results: Cell viability measured after isolation was: LC - 76±4%, MMSC – 94±2%. On the 30th day after BLU transplantation GPT, GOT, ALP returned to normal levels. In control rats, the same indices returned to normal levels more than in 6 months. A viable and high proliferative activity of transplanted LC was determined more than in 180 days after BLU transplantation.
Conclusions: Our studies asserted the long-time survival of LC and MMSC attached on matrices and transplantated into damaged livers. We consider that the present data are an important step toward the clinical application of intracorporeal BLU as a bridge to OLT.

P14 (EI0011)
THE *EX VIVO* NORMOTHERMIC PERFUSED LIVER-KIDNEY MODEL: AN IMPROVEMENT OF THE CIRCUIT BIOCHEMICAL AND ACID-BASE ENVIRONMENT
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Objectives: The *ex vivo* liver perfused model allows a better and unequivocal analysis of changes obtained by dissociating the organ from the extrinsic regulatory mechanisms. We now analyze the influence on the biochemical environment obtained with the addition of a kidney to the circuit.
Methods: Eight livers were harvested from female pigs and perfused for 6 hours. In five additional experiments a kidney was also harvested and connected in parallel. The extracorporeal circuits included a centrifugal pump, heat exchanger, and oxygenator. Hourly arterial blood gases were collected to analyze glucose, PH, bicarbonate, base excess, urea, creatinine, sodium, potassium. Primary end-point of the study was to evaluate the influence of the kidney on glucose, PH and electrolytes levels.
Results: In the liver-kidney circuit all parameters examined had significant lower values compared to the liver circuit only. This was particularly evident for glucose values where a normoglycemia was reached at the end of the perfusion and for PH and electrolytes that were maintained on steady levels.
Conclusions: The addition of the kidney to the circuit provides a better biochemical environment by filtering the excess products continuously released from the metabolisms. This could open the way for future experimental *ex vivo* models that require a strict balance of these elements.

P15 (EI0135)
INDICATIONS AND LIMITS OF THE REPLACEMENT THERAPY OF THE LIVER DETOXIFYING FUNCTION BY MARS (MOLECULAR ADSORBENT RECIRCULATING SYSTEM) IN THE TREATMENT OF ACUTE-ON-CHRONIC LIVER FAILURE (ACLF)
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Objectives: To assess indications and limits of MARS in ACLF treatment.
Methods: 98 patients affected by ACLF, secondary to different liver diseases, 80 with INR values < 3 (group A) and 18 with INR values ≥3 (group B,) have been treated with MARS (5 h daily sessions, blood flow 220±20 mL/min, albumin 150 mL/min, sessions from 2 to 7, according to the patien's needs). All the measured liver function parameters were not significantly different between the two groups. 42 patients lamented severe pruritus and showed scratching skin lesions before the treatment.
Results: At the end of each treatment total bilirubin, bile acids and ammonia fell of 28±9%, 40±8%, 54±14% respectively, with post-treatment rebound variable in each subject. After a cycle of MARS treatments liver function tests improved, total bilirubin, bile acids, ammonia, alkaline phosphatase and INR values significantly decreased in group A, while no improvement was observed in group B. Pruritus disappeared in all the patients after the third MARS treatment.
Conclusions: These observations suggest that the severe lack of coagulation factors, which reflects the end-stage liver failure, is the main condition indicating the exclusion of MARS treatment. The treatment is not absolutely contra-indicated even if other parameters are seriously altered. In fact its efficacy is strictly related to the potential recovery of liver function, independently of primary liver disease.

P16 (EI0125)
OBTAINING PIG LIVER SCAFFOLDS: DEVELOPMENT AND FIRST RESULTS OF A DECELLULARIZATION MODEL
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Objectives: Based on previous experiences published on decellularization of small animals' solid organs, in order to obtain bioenginered scaffolds for posterior recellularization, we aimed to develop a model to reproduce the aforesaid results in large mammals, as a previous step towards the translation to human liver. To achieve this goal, we propose a pressure controlled perfusion system for isolated organs with continuous monitoring. Using a detergent solution previously proved effective, we intend to obtain ten specimens.
Methods: We harvested ten specimens of minipig liver, using the same technique as in humans, with cold perfusion of preserving solution via portal vein and through hepatic artery via aorta. After the back table preparation, the organ was connected to the pressure controlled perfusion system, to continuously infuse a SDS based decellularizating solution for 24 hours. The system included a remote controlled pump with a pressure sensor all connected to a computer with the controlling software, developed at our institution. Once the decellularization procedure was finished, we checked the integrity of the vascular and biliary anatomy with radioscopy. A complete histological study was then performed, including optical and electron microscopy. Finally we determined the DNA residue present in the scaffold to assess the completeness of the lavage.
Results and Discussion: After a preliminary experience with three organs, we were able to establish a method for the decellularization. With this model, we performed seven more procedures with pig livers, which were all completely devoid of cells and comparable in terms of macroscopic appearance, histological and anatomical analysis and DNA residue.
Conclusions: We have developed a reproducible model for decellularizating large mammals' organs to use as bioengineered scaffolds for recellularization, which is a feasible initial step towards the aim of creating bioartificial organs for transplantation in humans.

P17 (EI0058)
MEMBRANES WITH EMBEDDED SORBENTS FOR AN ARTIFICIAL KIDNEY
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Objectives: Chronic kidney failure requires an artificial kidney treatment called hemodialysis. However, not all uremic toxins can be removed by dialysis. Sorbents can be used for the purification of blood, but this is often limited because of poor hemocompatibility. In this work, we propose a new concept to improve blood purification as well as the hemocompatibility when sorbents are used. We develop mixed matrix membranes (MMM) which consist of adsorptive particles embedded into a porous polymer matrix. The MMM can combine diffusion and adsorption in one step. To enhance the hemocompatibility of the MMM, a particle-free layer is introduced in the blood-contacting side of MMM, and several matrix materials are tested.
Methods: Porous MMM with activated carbon particles and a particle-free blood contacting layer were prepared by co-casting and spinning and subsequent phase separation. Several co-polymers with enhanced hemocompatibility as well as polyethersulfone were used as matrix materials. The membranes were characterized by scanning electron microscopy and adsorption experiments and screened for hemocompatibility.
Results: Double layer membranes with a homogenous distribution of carbon particles were developed. Creatinine was used as model blood toxin. The experiments show that creatinine diffuses through and adsorbs onto the MMM at the same time. Markedly, no quick particle saturation occurs. The contribution of the removal by adsorption is more than 80% of the total creatinine removal after 7 hours. The use of copolymers with hydrophilic and hydrophobic parts as matrix material leads to membranes with increased hemocompatibility.
Conclusions: This study shows proof of principle for MMM as an artificial kidney. MMM remove toxins via both diffusion as well as adsorption in one step. Our concept is versatile since we can combine various matrix materials with

improved hemocompatibility and various sorbents for the removal of toxins from blood.
Acknowledgements: *The DutchKidneyFoundation is acknowledged for financial support.*

P18 (EI0234)
MICROBUBBLES OF AIR DURING HEMODIALYSIS - NEGLIGIBLE FOR THE PATIENT?
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The symposium covers information about the presence of microbubbles in hemodialysis. Physical basis, technical considerations and regulations will be presented by Hans-Dietrich Polaschegg (Koestenberg, Austria). Per Jonsson (Umeå, Sweden) will cover experimental projects that were focused to evaluate the presence of microbubble air as contamination in the *in vitro* setting and some experiences. Those data and techniques were further used in *in vitro* testing of prevailing materials and basis for initial clinical studies on chronic hemodialysis sessions (Bernd Stegmayr, Umeå, Sweden). The symposium will thereafter focus on more clinical studies to investigate various clinical settings in relation to micro bubble exposure. This will be presented by Ulf Forsberg (Skellefteå, Sweden). The importance of microbubbles of air may be questioned. Therefore post mortem investigations were performed on hemodialysis patients. These data will be presented by Thomas Brännström (Umeå, Sweden). The symposium will end with time for discussion.

P19 (EI0160)
NANOPARTICLES OF CA; MG.CA AND ZN,CA HYDROXYAPATITE AS A COMPONENT OF FERROMAGNETIC POLYMER MATRIX
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Objectives: Ca_{10}; Mg_1Ca_9 and Zn_1,Ca_9 hydroxyapatite (HAP)- nanoparticles are proposed as a component of 3-D polymer matrix. Moreover, the incorporation of magnetic particles enables cell manipulation by an externally applied magnetic field. The adhesiveness and magnetic properties of matrix materials as one of the major conditions for the surviving of cells is considered. Process of forming the most suitable matrixes for cells in system polymer - HAP -ferromagnetic is studied.
Methods: Ca_{10}; Mg_1Ca_9 and Zn_1Ca_9 $(Po_4)_6$ $(OH)_2$ [HAP] particles are obtained by co-precipitation method in water solutions of salts and then dispersed in melt of polyethylene The analysis of matrices is carried out by XRD, FTIR. The adhesive properties of the matrix are studied using the estimation method of bond strength with metals at scaling. The measuring in magnetic field (2,5 kE) is carried out using magnetometer. The estimation of adhesion and growth of cells to surface of matrices is carried out with the use of a light microscopy, TEM, SEM and fluorescent microscopy. The morphology and viability of fibroblasts cells and cells of a hepatoma of human HepG2 on the surface of matrixes are studied.
Results: The samples possess an amorphous structure of HAP, or incompletely crystallized with additional peaks of polymer. The size of nanoparticles is in the range from 10.0 to 60.0 nm.The adhesiveness of matrix to metals varies from 2.0 to 14.0 kg/cm. Magnetization of matrices varies from 7.5 to 23 emu and concentration of magnetic phase from 4.0 to 12.0%. The visualization of cells on the matrix surface allows concluding that all samples are not toxic. The adhesiveness of cells to a surface is very different. The characteristic properties of ells depend on the composition of matrix.
Conclusions: The received data have practical significance for reconstructive surgery and transplantation.

P20 (EI0260)
PREPARATION AND CHARACTERIZATION OF WATER-SOLUBLE C60/SILK FIBROIN NANOCOMPOSITE FOR CARTILAGE REGENERATION APPLICATION
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Objectives: Studies have showed the role of water-soluble C_{60} in protection of articular cartilage against progression of osteoarthritis. Silk fibroin-based scaffolds also have been explored in cartilage or bone tissue engineering for years. Among them, aqueous derived silk fibroin scaffolds prepared via salt-leaching approach acted as promising candidates in tissue engineering application. However, salt-leached silk fibroin scaffolds derived from highly concentrated aqueous silk fibroin solutions have not been reported. In this study, the aim is to prepare aqueous derived salt leached silk fibroin scaffolds with improved mechanical properties. Furthermore, these novel scaffolds will combine with water-soluble C_{60} to generate nanocomposites for cartilage regeneration.
Methods: Silk fibroin was firstly extracted from silkworm *bombyx mori* by degumming in sodium carbonate solution. Then, the silk fibroin was dissolved in lithium bromide solution and dialyzed against distilled water. By its turn, concentrated silk fibroin solution was achieved by dialysis against poly(ethylene glycol) solution. Salt-leached silk fibroin porous scaffolds were prepared by the addition of sodium chloride particles into the silk fibroin solution. Water-soluble C_{60} was prepared via acid treatment and then incorporated into silk fibroin solution to prepare the nanocomposite scaffolds. The physicochemical properties of the silk C_{60} scaffolds were characterized.
Results: The mechanical properties of the silk fibroin scaffolds improved dramatically when prepared with high concentration silk fibroin solutions. The FTIR and NMR spectra showed that the carboxyl group and methacrylate group was successfully grafted with $C_{60.}$
Conclusions: A novel salt-leached silk fibroin scaffold was generated by using highly concentrated silk fibroin solutions. The water-soluble C_{60} can be prepared via chemical modification. It is expected that the preparation of water-soluble C_{60}/silk nanocomposite could bring new insights in cartilage regeneration.

P21 (EI0092)
MSCS PROLIFERATION AND OSTEOGENIC DIFFERENTIATION ON 2D AND 3D PCL NANOFIBROUS SCAFFOLDS
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Objectives: Nanofibers appear to be the ideal material for scaffold development in regenerative medicine. On the other hand, electrospun nanofibres form two-dimensional (2D) net. Cells can proliferate only until confluence is reached. In this work advantages of extensive surface and three-dimensional (3D) scaffolds were mixed together to prepare 3D electrospun nanofiber scaffold.
Methods: Two different samples were prepared from PCL using electrospinning in the same conditions, only the collector was different. In the case of 2D nanofibers, the collector was plain, to prepare 3D samples, a structured collector was used. The structure of samples was visualized by scanning electron microscopy (SEM). Samples were seeded with 9×10^5 pig MSCs and cultured for 21 days. For detection of MSCs adhesion, spreading area of cell was measured. Cells were stained using DiOC and visualized by confocal microscopy. Proliferation and viability were detected using MTT assay and live/dead staining with subsequent confocal microscopy visualization. Osteogenic differentiation was investigated with real-time PCR analysis; osteocalcin (OC) and bone sialoprotein (BS) were used as osteogenic markers.
Results: Both, structured and non-structured samples were successfully prepared. SEM shows that samples prepared using structured collector have 3D structure. MSCs adhered well on both, 2D and 3D scaffolds. On 3D scaffolds spreading area was slightly higher. Proliferation was higher on 3D nanofiber scaffolds on 21 day. Better proliferation was confirmed by live/dead staining and confocal microscopy. OC and BS were used as markers for detection of late osteogenic differentiation. In 2D and 3D samples osteogenic markers were present, whereas higher amount of both markers were shown on 3D scaffold on 21 day.
Conclusions: It was shown that 3D structured PCL nanofibers are promising for the purposes of tissue engineering.
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P22 (EI0149)

PLASMA EXCHANGE IN ANCA-ASSOCIATED VASCULITIS WITH SEVERE RENAL INVOLVEMENT

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Objectives: Systemic vasculitis associated with autoantibodies to neutrophil cytoplasmic antigens (ANCA) is the most frequent cause of rapidly progressive glomerulonephritis. Renal failure at presentation carries an increased risk for chronic kidney disease (CKD) and death despite immunosuppressive therapy. Early and accurate diagnosis and aggressive treatment are essential to optimizing outcomes while avoiding unnecessary immunosuppressive therapy.
Methods: This study investigated the role of plasma exchange in the achievement of renal recovery in patients who presented a serum creatinine 500 µmol/L. We present here three patients treated in ICU at the University Clinic of Nephrology in Skopje, with confirmed diagnosis of ANCA vasculitis associated with respiratory symptoms and a renal affection manifested as glomerulonephritis.
Results: All patients had diagnosis of ANCA-associated vasculitis confirmed by renal biopsy and serum creatinine >500 µmol/L. Initial hospital admission was marked by serious respiratory symptoms with the development of important deterioration of the renal function as well as anemia and hypoproteinemia. Dialysis treatment was introduced, as well as immunosuppressive therapy, with prednisolone and cyclophosphamide by EUVAST recommendations. Plasma exchange was also introduced in at least 9 sessions per patient. This, together with immunosuppressive therapy, resulted in a disappearance of signs and symptoms of systemic inflammation and in an important improvement of respiratory symptoms and moderate improvement of kidney function. Patients were discontinued from dialysis and at a point of 3 months after admission all patients were dialysis-independent.
Conclusions: In patients with clinically and histologicaly confirmed ANCA-associated vasculitis, plasma exchange together with recommended immunosuppressive therapy can increase the rate of renal recovery and should be considered as an effective adjunctive modality of treatment.

Smart and Responsive Biomaterials

P23 (EI0348)

SYNTHESIS OF POLY(ACRYLAMIDE-CO-ITACONIC ACID) HYDROGELS AND THEIR INTERACTIONS WITH CALCIUM IONS AND ANTIBIOTIC WITH POTENTIALS APPLICATIONS IN ORTHOPEDIC MEDICINE

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Objectives: The need to repair bone defects is a significant problem faced in orthopedic medicine. Biomaterials, such as polymers are used in bone tissue engineering. Calcium phosphates, used clinically in orthopedic surgery, have attracted attention as bone substitutes, due to their good biocompatibility and osteointegrative properties[1]. In this work hydrogels obtained from poly (acrylamide) and poly (acrylamide-co-itaconic acid) were synthesized and characterized to determine their capacity for calcium absorption and antibiotic interaction.
Methods: Hydrogels copolymers of acrylamide (AAm)/itaconic acid (AI), crosslinked with N,N-methylenbisacrylamide were synthesized, from AAm/AI with fed proportions 90/10 and 80/20. The hydrogels swelling degree was measured in deionized water and in calcium salts solutions; also, it was measured in antibiotic (Tygacil). Inductively coupled plasma optical emission spectrometer (ICP-OES) was used to analyze the aqueous calcium solutions. Hydrogels were observed by scanning electron microscope (SEM) (Hitachi S-800). By SEM it was also analyzed the calcium absorbed in the hydrogels structure by EDX.
Results: In poly(AAm-co-AI) hydrogel the equilibrium swelling measured in aqueous calcium solutions decreased between 5 to 10% compared with the swelling in pure water. ICP-OES results indicates that poly (AAm-co-AI) hydrogels have an efficient absorption toward calcium ions (13,69 mg Ca^{2+}/g hydrogel), while poly(acrylamide) only absorbed 3.83 mg Ca^{2+}/g hydrogel; then, they were submerged for 24 h. The poly(acrylamide) hydrogels morphology evaluated by scanning electron microscopy revealed pore dimension ranging from 210 nm to 1430 nm, which increased to 6900 nm when it was copolymerized with the itaconic acid.
Conclusions: The results show evidence of an efficient reception of Ca^{2+} ions by the polymers, especially poly(AAm-co-AI) hydrogels containing 20% of AI. The porosity morphology revealed interconnected pores, which is important because it has been reported that an open porosity would be necessary to ensure rapid colonization of the implant with blood vessels and bone cells.

P24 (EI0178)

DEVELOPMENT OF AN ELECTROCHEMICAL SENSOR FOR HEPARIN IN BLOOD USING MOLECULARLY IMPRINTED POLYMER

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Objectives: Inhibition of blood-coagulation is very important for safe extra-corporeal circulation. However, suitable methodology for monitoring anticoagulant in blood is yet to be established. The purpose of this study is the development of a sensor for the level of heparin, which is the well-used anticoagulant, using an electrode grafted with molecularly imprinted polymer (MIP).
Methods: A photoinitiator of radical polymerization was introduced on indiumtin oxide (ITO) covalently. Heparin and (2-mathacryloxyethyl) trimethylammonium chloride and acrylamide was dissolved in water. Methylenebisacrylamide was dissolved in dimethylformamide. The initiator-immobilized ITO was soaked in the mixture of the solutions and was irradiated by UV-lump for graft polymerization. The treated electrode was rinsed by water to obtain an electrode grafted with heparin-imprinted polymer (HIP). Another electrode grafted with non-imprinted polymer (NIP) was prepared by the same procedure except heparin was omitted. A traditional cyclic voltammetry of ferrocyanide was performed with the polymer-grafted electrode in physiological salt solution diluting bovine blood. The effect of heparin in blood on the oxidation current at the voltammetry was observed.
Results: The anodic current at the HIP electrode was increased by the presence of heparin in the blood (4 unit/mL). The change of current by heparin was reversible. However, the current at the NIP electrode was insensitive to the heparin. The current change at HIP was probably due to specific binding between heparin and the imprinted site, which was created during the polymerization. Then the porosity of the HIP layer was increased responding to the specific binding and accessibility of the ferrocyanide toward electrode was enhanced.
Conclusions: The concentration of the heparin in the blood can be detected by voltammetry using an electrode grafted with heparin-imprinted polymer. The procedure is very simple. Then the method is feasible for monitoring heparin in the circulated blood.

P25 (EI0311)

PREPARATION OF BIOINSPIRED MAGNETIC RESPONSIVE HYDROGEL PARTICLES FOR BIOMEDICAL APPLICATIONS USING SUPERHYDROPHOBIC SUBSTRATES

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Objectives: In tissue engineering and regenerative strategies the number of cells extracted from the patient are not enough for the therapy. In this context, polymeric microspheres have been used as supports for the expansion of cells. Recently inspired by the rolling water droplet on a lotus leaf, a novel methodology on preparing hydrogel and polymeric spheres was developed using superhydrophobic substrates. In this contribution, magnetic responsive hydrogel beads were prepared by this innovated method; furthermore, the cells attachment was investigated on these hydrogel beads before and after plasma treatment. The introduction of magnetic microparticles during sphere production permits the isolation of the particles from the culture medium simply by using an external magnetic field.
Methods: Polystyrene superhydrophobic substrates were prepared by a simple phase inversion method. Magnetic microparticles of Fe3O4 were introduced during hydrogel sphere production process. After frozen dried, the beads were further treated by Argon plasma. Cells attachment was investigated on both treated and untreated ones.
Results and Conclusions: Magnetic responsive chitosan hydrogel spheres

crossing-linked by genipin were hardened on the superhydrophobic surfaces. Upon plasma treatment, cell attachment onto the beads surface was improved as compared to the untreated ones. The chitosan beads could move throughout the liquid medium by the action of an external magnetic field. After extracting the particles from the medium the cells could be detached from such supports by the action of trypsin and the particle could be used again. The extracted cells were found to maintain their viability. In conclusion, magnetic responsive hydrogel beads could be prepared by using superhydrophobic substrates

P26 (EI0303)

HR-MAS NMR SPECTROSCOPY AS EFFICIENT TOOL TO CHARACTERIZE CROSSLINKED HYDROGELS

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Objectives: In the present work, high-resolution magic angle spinning (hr-MAS) NMR spectroscopy is applied as a straightforward non-destructive technique to quantify unreacted methacrylamide functionalities in cross-linked gelatin hydrogels.

Methods: 10 w/v% cross-linked methacrylamide-modified gelatin (gel-MOD) hydrogel films were prepared and lyophilised, followed by resuspension in D_2O. The effect of the photo-initiator concentration (Irgacure 2959, 0.5–10 mol%) and the applied UV irradiation time (5–30 minutes) on the consumption of methacrylamide moieties was evaluated using hr-MAS NMR spectroscopy.

Results: The results (data not shown) indicate that a critical amount of 2 mol% photo-initiator is required to obtain a significant amount of methacrylamide-crosslinking. Upon increasing either the photo-initiator concentration (2–10 mol%) or the UV irradiation time (5–60 min), the percentage of reacted methacrylamides increased significantly ($P<0.05$). Interestingly, it can be observed that even at a UV irradiation time of 1 hour and a photo-initiator concentration of 10 mol%, only 40% of the methacrylamide side groups have reacted. In a final part of this work, rheological measurements were performed to correlate the mechanical properties of the hydrogels developed with the cross-link efficiency obtained from hrMAS NMR. Increasing either the photo-initiator concentration or the UV irradiation time, leads to an increased storage modulus. In addition, the mechanical data indicated that both the storage and loss moduli display a pronounced plateau value in the frequency region studied. Moreover, G' is about two orders of magnitude higher than G", which is indicative for the formation of a well-established network.

Conclusions: We can conclude that hr-MAS NMR spectroscopy is a suitable, non-destructive and straightforward tool to evaluate absolute hydrogel crosslinking efficiencies. Although the technique was only applied for gelatin-based hydrogels, we are at present investigating the applicability to other hydrogels including vinyl functionalised Pluronic-based systems.

P27 (EI0205)

ENHANCED FUNCTION AND ADHESION MECHANISM OF HUMAN BONE MARROW CELLS ON FUNCTIONALIZED CARBON NANOTUBES

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Objectives: Carbon nanotubes (CNTs) have attracted much attention as a biomaterial with interesting potential applications. The disadvantage of insolubility has been overcome by functionalization of CNTs surface. In this study, human bone marrow stromal cell (HBMSC) adhesion, proliferation, and differentiation, cultured on multi-walled CNTs, either pristine or amino-, carboxy- and hydroxy-functionalized, were investigated. Preliminary results on integrin-mediated adhesion mechanisms were also obtained.

Methods: Functionalized CNTs were synthesized according to well-established methods and were fully characterized with TGA and Raman analysis. The HBMSCs were routinely cultured in human osteogenic medium. Cells of second to forth passage were seeded on the materials. Morphology and adhesion were evaluated by SEM after 1 and 3 days, proliferation was estimated by DAPI staining after 3 and 7 days and differentiation through alkaline phosphatase activity (ALP). To screen which integrins were responsible for attachment of the cells, monoclonal antibodies against integrin subunit β_1 were incubated with the cells for 30 min at 37° C, prior to seeding on the CNTs surfaces.

Results: SEM images display that HBMSCs attach and spread well on all CNTs surfaces without differences in their morphology. Proliferation was highest on amino-functionalized CNTs, but not significantly different from the other surfaces. However, proliferation on the control (tissue culture plastic) was 3-fold. The highest expression of ALP activity was on hydroxy-functionalized CNTs and the lowest on pristine. After 3 days of culture, the ALP expression on CNT surfaces was approximately 70% of the control, whereas after 7 days, it was 4-fold of the control. The blocking of integrin subunit β_1 with monoclonal antibodies resulted in a decrease of the adhesion percentage to pristine CNTs at 40-50%.

Conclusions: It is possible that nanomaterials, whose structural features resemble those of natural tissue, enhance cell function and CNTs can be used as orthopaedic biomaterials.

P28 (EI0124)

PHYSICAL CROSSLINKING OF GELATIN: A SUPRAMOLECULAR APPROACH TO BIOMATERIAL

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Objectives: Key properties that a biomaterial should address include elastic properties close to the substituted tissue, specific adhesion epitopes, and tailorable degradability. A knowledge-based approach formed the basis for investigating the systematic variation of material properties of gelatin by introducing functional groups derived from tyrosine for enabling π-π interactions as well as hydrogen bonds to form stable physically crosslinked networks.

Methods: Gelatin was functionalized with desaminotyrosine (DAT) or Desaminotyrosyl-tyrosine, (DATT). Atomistic molecular models of pure and functionalized gelatin with 0.8wt.-% or 25wt.-% water content were constructed using Material Studio (Accelrys) and submitted to the Amorphous Cell module to create bulk packing systems. The dynamic behaviour, structural, and mechanical properties were investigated by analyzing free volume distribution, solubility parameters, elastic properties, and aggregation phenomena. The functionalized gelatins were synthesized by coupling of the free carboxylic acid groups of DAT(T) to the amino groups of gelatin and the materials were characterized by tensile tests, TM-DSC, swelling experiments, and WAXS.

Results: The simulations predicted an increasing number of aromatic functions attached to the gelatin chain leading to an increase in the number of physical net-points. In the synthesis, about 80mol.-% of all amino groups were functionalized with DAT(T). Increasing the number of aromatic groups attached to the gelatin chain resulted in suppression of helix formation and decreased the swelling degree. Mechanical properties (Young's modulus, elongation at break, and maximum tensile strength) of the gels at equilibrium swelling increased with the number of introduced aromatic groups.

Conclusions: Distinct tailoring of material properties was achieved for a biopolymer by only small changes in molecular structure of gelatin. The approach of molecular modelling of gelatin as bulk material permits to analyze structural features of functionalized materials and can be used as predictive tool in the design of new biopolymer-based materials.

P29 (EI0336)

TEMPERATURE-RESPONSIVE MICROCAPSULES PREPARED BY NANO-STRUCTURED MULTILAYERS OF CHITOSAN AND AN ELASTIN-LIKE RECOMBINAMER FOR THE CONTROLLED RELEASE OF THERAPEUTIC MOLECULES

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Objectives: Polyelectrolyte vesicles using layer-by-layer (LbL) were recently introduced for the encapsulation of therapeutic molecules. This work presents multilayered microcapsules of chitosan and a temperature-responsive elastin-like recombinamer (ELR) as a novel drug delivery system. The release of a pre-loaded model protein was studied at distinct temperatures and number of layers to evaluate the permeability of these structures and their potential as tunable drug delivery devices.

Methods: Sacrificial $CaCO_3$ microparticles were prepared by co-precipitation of Na_2CO_3 and $CaCl_2$ in a FITC-BSA solution under heavy stirring. LbL coating was performed by incubation with chitosan or ELR solutions, with a rinsing step in between. Capsules with 1, 3 and 5 bilayers were made. The $CaCO_3$ cores were chelated using EDTA. The capsules were suspended in PBS at 25 and 37ºC and samples were taken every 24 hours for fluorescence measurements, during 14 days.

Results: At both temperatures, cumulative release was higher for capsules with 1 bilayer, evidencing the role played by the capsules architecture in their permeability. The release kinetics among each temperature was also different: the BSA quantity released was higher at 25ºC than at 37ºC. Considering the case of a simple bilayer, in the former the cumulative release reaches 80%, while in the latter only 50% of the encapsulated protein is released. This result shows the effect of temperature in polyelectrolyte structures, namely when temperature-responsive materials like ELRs are used.
Conclusions: Multilayered microcapsules based on chitosan and an ELR were studied as drug delivery vessels. Distinct release profiles of pre-loaded BSA at different temperatures and layer numbers demonstrated the influence of the capsules architecture and composition: more quantity of BSA is released for capsules with fewer layers and lower temperatures. These microcapsules have the potential for tunable drug release in tissue engineering applications by means of design changes.

Engineering for Cardiac Assist Devices

P30 (EI0380)
CFD ANALYSIS OF THE BLOOD FLOW IN A HOLLOW FIBER MEMBRANE OXYGENATOR WITH MULTIPLE PASSAGEWAYS
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Objectives: This work presents a 3D computational fluid dynamics (CFD) approach for modelling blood flow in a state-of-the-art hollow fiber membrane oxygenator with integrated heat exchanger. During extra-corporeal circulation blood needs to be heated and oxygenated before being reinfused into the patient. Optimal gas exchange and minimal pressure drops are two basic requirements in the design of membrane oxygenators. In the present study, the effects of multiple passageways to enhance blood oxygenation within the device were assessed through a CFD analysis.
Methods: The fluid volume inside the device was discretized with about 2.5 million elements. Blood was modeled as an incompressible Newtonian fluid with viscosity μ=3.0 mPa·s and density ρ= 1060 kg/m^3. A blood flow of 4 L/min at the inlet section gave rise to non-laminar inflow conditions (Re=2880). Hence, the viscous k-ω two-equations turbulence model with low Reynolds number corrections was adopted. The heat exchanger and oxygenator regions were modeled as porous media with permeability values obtained from experimental tests. Laminar flow regime was assumed in those regions. CFD simulations were performed using the commercial software ANSYS FLUENT®.
Results: Computational results were post-processed to extract the flow velocity pattern, the potential stagnation areas and the pressure drops. The shape of the oxygenator ensured a good intermixing of flow thanks to the multiple passages of blood inside the fiber bundle. The overall pressure drop was equal to 200 mmHg. High local pressure gradients, originating from high-flow concentrations in collecting and distributing regions, were detected.
Conclusions: The CFD-aided analysis allowed evaluating advantages and drawbacks of the device geometry. The tortuous pattern of blood inside the oxygenator may be an effective strategy to enhance mass and heat transfer within the device, by allowing a multiple blood crossflow within the fibre bundle at low velocities and limiting the pressure drops.

P31 (EI0252)
TRANSCUTANEOUS ENERGY TRANSFER SYSTEM (TET): *IN VITRO* AND *IN VIVO* VALIDATION
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Objectives: Percutaneous drivelines cause infections and technical problems. To minimize complications and increase patient's mobility, a transcutaneous energy and data transfer system is to be developed with high tolerance of transmission and a convenient external carrier system.
Methods: The inductive TET includes two coreless coils (D=60mm) with external and internal control units equipped with accumulators. An integrated controller provides telemetry data processing and control of the implant. Wireless data transfer is enabled by using RF transmission and a proprietary protocol. The performance is verified in a body simulator *in vitro* and in acute animal studies (pigs, n=4). A positioning assistance is developed for exact placement of the external coil. A carrier system for the external components is designed as a flat textile backpack in which the external transmitter coil is integrated. The carrier system is verified in a study with VAD patients.
Results: The developed TET is able to transmit up to approx. 25 Watt through the tissue. Bi-directional data communication is improved to a rate of 500 kbits/sec, were the external receiver is allowed to be up to 3m distant to the patient. The maximum efficiency of the system is approx. 83% at 15mm distance between the coils and 79% at 25mm distance. Displacement of the coils up to 20mm reduces the efficiency up to 15% and leads to a warming of the external transmitter electronic. No warming is measured between the coils and the implanted components under any operating condition. The positioning system enables easy alignment of the external coil with an accuracy of 1.6mm.
Conclusions: The TET shows reliable transmission at horizontal and vertical displacements up to 35mm. Transmitted energy is automatically adapted to the demand of the implanted device. Twisting of the flexible coils did not influence the transmission appreciably.

P32 (EI0167)
DEVELOPMENT OF A PORTABLE PNEUMATIC DRIVER FOR THE WHOLE RANGE OF BERLIN HEART EXCOR BLOOD PUMPS
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Objectives: Paracorporeal, pneumatically driven VADs are preferably used for biventricular adult support as well as for pediatric patients. For the EXCOR® System (Berlin Heart GmbH, Germany), a new pneumatic driver that combines the performance and versatility of the existing stationary driver with the flexibility of the existing mobile driver is currently under development.
Methods: The new driver uses the reliable and proven pneumatics technology of the EXCOR mobile driver. Two piston pumps operate in synchronism with the blood pumps. The piston of each pump is driven by an electric motor via a ball-screw. A closed pneumatic system was chosen to optimize the system efficiency. A control system regulates the pneumatic pressure and blood flow waveform, emulates the Frank-Starling behavior, synchronizes both blood pumps and ensures optimal adjustment of the enclosed air mass. An emergency mode of operation is provided by a fault-tolerant embedded computing system in combination with a dedicated crossover valve without unduly increasing the system complexity and weight. Special attention has been paid to easy handling and a clear alarm and message structure. The driver is mounted on a cart and is equipped with two easily exchangeable batteries, a mains power adapter and a car power adapter.
Results: At 8kg the new driver is lighter than the EXCOR mobile driver. It supports blood pumps in the range of 10mL to 80mL. The power consumption for biventricular adult support is 20W and less for pediatric support. The batteries guarantee cordless operation for at least 8 hours. Bench tests have demonstrated correct performance with respect to preload sensitivity and flow profile.
Conclusions: The new pneumatic driver is able to drive all sizes of EXCOR blood pumps. Its small dimensions, low weight and new design make it suitable for stationary as well as portable use.

P33 (EI0148)
THE CONTINUOUS FLOW LVAD WITH NATIVE HEART LOAD CONTROL SYSTEM (NHLCS) FOR BRIDGE TO RECOVERY COULD CONTROL THE CORONARY FLOW AND MYOCARDIAL OXYGEN CONSUMPTION IN ACUTE HEART FAILURE MODEL
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Objectives: A novel control system for continuous flow LVAD has been developed for bridge to recovery. We have reported that the amount of coronary flow and myocardial oxygen consumption (MVO2) could be controlled by changing its rotation speed in synchronization with the native cardiac cycle, in normal heart models. We will confirm whether the coronary flow and MVO2 can be controlled by the NHLCS under acute heart failure conditions.
Methods: Ten adult goats (61.4±12.6 kg) with acute LV dysfunction due to coronary microsphere embolization (50µm, 0.42±0.22million) to left Anterior descending artery were used for the experiment. The continuous flow LVAD

(EVAHEART) was installed via left thoracotomy. Blood uptake was from Apex and return it to descending aorta. Ascending aortic flow, pump flow, coronary flow of the left main trunk were monitored. LV volume and pressure were also monitored. We performed 4mode, Circuit clamp (no support), Continuous (constant rotation), Counter pulse (increase rotation in diastole), Co pulse (increase rotation in systole) with the 100% bypass rate.
Results: The amount of coronary flow in counterpulse mode was proved to be significantly increased than any other modes [Continuous/Counterpulse/Copulse: 114.0±2.7/121.8±2.7/101.6±2.7 ($p<0.05$)]. We also find out that MVO2 was decreased in counter pulse mode and increased in Copulse mode compared with continuous mode [Continuous/Counterpulse/Copulse: 79.7±13.9/70.2±19.2/88.5±13.8 ($p<0.05$)] (circuit-clamp mode=100)
Conclusions: We have showed the possibility for changing the coronary flow and MVO2 (=heart load) by controlling the rotation of continuous flow LVAD. It may be suitable to use counterpulse mode for support mode by its large coronary flow and small MVO2. And it may be also suitable to use copulse mode for heart training mode by its large MVO2. Using this novel control system (NHLCS) flexibly according to patient circumstances, may contribute to the bridge to recovery therapy for patient with LVAD.

P34 (EI0227)
DEVELOPMENT OF AN INNOVATIVE MOCK CIRCULATORY LOOP FOR VAD TESTING
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Objectives: In this paper a novel approach for the design of a Mock Circulatory Loop (MCL) is presented. This modular MCL concept is composed of active components only that are integrated into a Hardware-In-The-Loop (HIL) simulation. Hence, various conditions of a patient and transitions between conditions can be simulated with the MCL.
Methods: At present the newly developed MCL consists of two modules. The modules are composed of active components enabling optimal controllability of the system. In its current configuration it can be used to test ventricular assist devices. As vessels are typically described as a combination of a resistance and a compliance the modules are composed of gear pumps and metal bellows that are actuated from voice coil actuators. They represent the vessel resistances and compliances. The inertance of larger vessels was neglected. Furthermore, the MCL can mimic the heart as well, because ventricles are usually modelled as variable compliances. Since the MCL is part of a HIL-simulator, it is controlled by a dSpace DS1103-system that allows real-time simulation of the cardiac circulation. A software simulation of the cardiovascular system was used to generate setpoint values for the modules.
Results: At first, measurements with the components of a module were performed in order to verify compliance with the prior defined requirements. Afterwards, simulations of the aortic and left ventricular pressure were used as setpoint values for the modules. Both modules successfully met the requirements and were capable to trace the desired hemodynamic conditions.
Conclusions: The results demonstrate the feasibility of the test stand. In a next step the MCL will be enhanced by two additional modules for total artificial heart testing.
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P35 (EI0137)
APPLICATION OF A HYBRID MODEL TO CONTINUOUS FLOW PUMP INVESTIGATION
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Objectives: Continuous flow pumps (CFP) are widely used and differ from each other in types, connection and performance. Circulatory and ventricular conditions too play a role in pump performance. The number of variables involved makes modelling an appealing tool to study pump performance in different patient's conditions and to support clinical decision. This work aims at developing a circulatory model merging a computational model (CM) with a CFP working in its own environment. In this way it is possible to investigate the CFP effects on variables calculated by the computational model but influenced by the pump.
Methods: The lumped parameter CM consists of left and right hearts, systemic, pulmonary and coronary circulation. The CFP is represented by an electrical model realised using operational amplifiers. The pump speed and the slope of the pressure-flow characteristics can be controlled to simulate different pump types. Pump performance was analyzed considering circulatory (Cardiac Output-CO, left atrial pressure-LAP, aortic pressure-AOP) and ventricular variables (ESV and EDV) against ranges of left ventricular E_{maxl} (0.5-2.5-3.5 mmHg·cm^{-3}) and stiffness (30-60 cm^3·mmHg^{-1}). All experiments were conducted comparing the selected variables before and after pump activation. The pump was connected between left atrium and aorta.
Results: Chosing maximum pump speed and E_{max}=3.5 mmHg·cm^{-3}, CO, LAP and AOP variations are remarkable: 45%, -300% and 73%, respectively. The pump flow shape is influenced by the pressure variations across the pump head. Different ventricular and circulatory conditions produce remarkably different effects and it is possible to identify dangerous situations (suction).
Conclusions: This work demonstrates the possibility to merge a CM with a device working in a different environment. The model provides a platform to produce stable and repeatable circulatory conditions. The CM will be connected to a real device using the technology developed in the frame of EU SensoART project.

P36 (EI0068)
A NOVEL IMPLANTABLE SENSOR TO MONITOR BOTH APICAL ROTATION AND CARDIAC PHASES
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Objectives: The magnitude and timing of Left Ventricular (LV) twist with respect to cardiac phases are essential to detect systolic and diastolic dysfunction, as recently shown by advanced imaging techniques. No implantable sensors are currently available to provide that phasic analysis of LV rotation over the cardiac cycle. We developed and evaluated in a sheep model an innovative implantable gyroscopic sensor for the continuous endocardial monitoring of both the amount and timing of cardiac rotation.
Methods: In a sheep, a tip catheter gyroscopic sensor was inserted in the endocardium of the right ventricle apex. The detected signal (EndoTwist) was continuously recorded along with ECG, LV pressure (LVP) and its first derivate (LVdP/dt). EndoTwist was processed in order to obtain both cardiac rotation parameters (twist rate ω, apical rotation angle θ) and mechanical heart vibrations (MHVs) used to identify systole and diastole.
Results: The detected EndoTwist signal clearly showed both a low-frequency component relating to cardiac rotation (ω, θ) and a high frequency component relating to MHVs. Identification of systole and diastole from MHVs was confirmed by comparison with LVdP/dt which was previously used to define the timing of the cardiac cycle.
Conclusions: The new implantable sensor permits detection of cardiac twist dynamics (ω, θ) with respect to the entire cardiac cycle by means of MHVs recognition. This information, if confirmed in larger studies, has promising clinical implication for the monitoring of cardiac function in heart failure patients.

P37 (EI0060)
THE ESTABLISHMENT OF THE QUANTITATIVE EVALUATION STANDARD FOR THE ANATOMICAL COMPATIBILITY OF THE VENTRICULAR ASSIST DEVICE
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Objectives: The body surface area (BSA) is currently used as a standard to determine the anatomical compatibility for patients with a ventricular assist device (VAD). However, it is difficult to accurately evaluate the anatomical compatibility for the patients when the patient's BSA measurement is close to the standard value of BSA. The purpose of this study is to establish a new quantitative standard that is more accurate than the currently used BSA standard.
Methods: BSA values of subjects were calculated using height and weight. The three-dimensional (3D) models of the chest and abdomen were constructed on the computer by means of the image processing software using the computerized tomography (CT) images of the chest and abdomen for subjects. The volumes of the chest and abdomen (VCA) were calculated by means of the 3D models. In this study, the relationship between the BSA and the VCA was examined for subjects who have the same BSA value.
Results: As an example, the resulting BSA and VCA measurements for two subjects showed that even though the BSA measurement was 1.6 for both subjects,

the VCA measurements of 61 and 53 revealed a large difference.
Conclusions: The VCA was evaluated as a new quantitative standard. The results of this study suggest that VCA give more detailed information to us for the standard to evaluate anatomical compatibility than BSA.

P38 (EI0084)
A NEW INTEGRATED HYBRID CARDIOVASCULAR SIMULATOR AS A SMART TOOL FOR VAD DEVELOPMENT, DESIGN AND RESEARCH
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Objectives: Why not to simulate VAD-Heart interaction easier and better? Pure physical cardiovascular simulators have many limitations, are not flexible, and do not reproduce precisely many parts of the circulatory system. Our main goal was to develop a VAD-Heart Simulation Platform (VHSP) – hybrid multipurpose tool enabling mechanical assist devices interfacing connection with a virtual (numerical model) circulatory system (VCS).
Methods: Four hydro-numerical impedance transformers (TR) integrated into one assembly were coupled with a VCS working in real-time. Each TR may be configured independently as an input or output channel connecting inflow or outflow cannula of VAD to the selected point of VCS. We can obtain a parallel or serial LVAD/RVAD assistance using two TRs. Four ones enable BVAD application. Adding an artificial valve, such as a mitral or aortic one, we extend a field of VHSP applications to e.g. valve's investigations.
Results and Discussion: VCS is seen from the VAD point of view as a physical model, but VCS is still numerical, so changing a computer software we can tailor a structure of VCS to the specific requirements. The change of any VCS parameter is only a few mouse clicks. Thanks to the TRs we can connect to VCS different pulsatile, nonpulsatile or centrifugal assist devices. Some experimental courses illustrating VCS performance are also included.
Conclusions: The presented VHSP is a result of a longstanding Polish-Italian group cooperation. Some applications like a parallel LVAD/RVAD/BVAD assistance are available. The next one will be released in near future. We are planning to enrich VCS introducing some physiological mechanisms, such as interventricular interaction, baroreflex feedbacks and cardio-pulmonary interaction. Potential VHSP applications are new VAD development as well as medical staff training.
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P39 (EI0231)
CFD-AIDED DESIGN OPTIMIZATION OF A CENTRIFUGAL BLOOD SEPARATING DEVICE
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Objectives: The purpose of this work is to define innovative strategies, based on advanced computational methods, to optimize the geometry and the functioning of a centrifugal hemoconcentrator. When autologous blood is retrieved from drainage during or after surgical operations, the device allows applying a centrifugal force field on blood in order to separate red blood cells (RBC) from plasma and from waste products. The separated portion of blood is then reinfused into the patient, thus avoiding the risks associated with allogenic blood transfusions.
Methods: Geometrical and functional optimization of a blood separator was performed by means of computational fluid dynamics (CFD) simulations. The transient process of apheresis was simulated with a multiphase mixture model in which two phases were defined: the granular RBC phase and the primary plasmatic phase. Blood was modeled as a Newtonian fluid; density values of 1030 Kg/m^3 and 1095 kg/m^3 were used respectively for plasma and RBC. A 33 mL/min flow was assigned at the inlet with an erythrocyte volume fraction of 0.1, and a centrifugal acceleration of 900 g was used. The maximum packing limit for the particulate phase was set to 0.7 in order to account for limited RBC deformability. Different design solutions were tested.
Results: CFD results in terms of RBC volume fraction and velocity fields within the device were compared among different geometries. This allowed assessing the hemoconcentrator efficiency working with a low hematocrit blood (Hct=10%) as inflow. Furthermore, the device performances were evaluated by varying the inlet flow rate, the rotational speed and the shape of the outlet conduits.
Conclusions: On the basis of CFD simulations results, new and improved geometrical configurations were proposed for the hemoconcentrator, in order to avoid vortex development and to increase the separation rate up to a 60% hematocrit concentrated blood at the outlet.

P40 (EI0255)
***IN VIVO* EVALUATION OF A WEARABLE PNEUMATIC TOTAL ARTIFICIAL HEART SYSTEM WITH A COMPACT DRIVE UNIT**
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Objectives: We have been developing a wearable Pneumatic Total Artificial Heart (PTAH) system with compact Wearable Pneumatic Drive (WPD) unit for bridge to transplant. This paper reports the current state of development including the results of the first acute animal experiment in PTAH system.
Methods: The PTAH system consists of left and right diaphragm-type blood pumps and two WPD units. Two blood pumps were designed to fit anatomy, and these had housings made of polyurethane resin and stainless steel. 25mm ID and 23mm ID Bicarbon valves were mounted in the inlet and outlet ports, respectively. The sizes of the left and right pumps are 95x68x49mm and 104x74x44mm. The stroke volume of the left and right pumps are 85 and 94mL. The WPD unit consists of a brushless DC motor, a crankshaft, a cylinder-piston, non-circular gears, and air pressure regulation valves. Driving air pressure is generated by the cylinder-piston. The non-circular gears generate the fixed systolic ratio. The size and weight of the WPD unit are 20x8.5x20cm and 1.8kg, respectively. The left and right pumps were implanted in a calf weighing 98kg. Two WPD units with fixed systolic ratio of 35 and 44% were connected to left and right pumps with 2m drive lines, respectively, and fundamental performance of PTAH system was evaluated in acute animal experiment.
Results: The cardiac output ranged between 3.9 and 5.4L/min at the mean aortic pressures of 108-115mmHg at pump rates of 60 to 100bpm. The average electric power consumption in each WPD unit varied from 5.3 to 17.6W according to the beating rate, and the efficiency was estimated to be 2-9%.
Conclusions: These results indicated that our PTAH system consisting of blood pumps and WPD units have sufficient performance for total cardiac replacement.

P41 (EI0328)
NUMERICAL METHOD OF FLOW STAGNATION AREAS ESTIMATION UNDER STEADY STATE CONDITIONS IN PNEUMATIC VAD
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Objectives: A numerical flow analysis of pneumatic VAD, POLVAD-MEV type in steady state conditions was performed. New numerical method of flow stagnation areas estimation in VAD was proposed. Results were compared with fibrin deposition detected in POLVAD-MEV pump after clinical applications (46 VADs used in four clinical center in Poland).
Methods: Flow simulations for two opposite operational states (end-diastole and end-systole) were simulated. In order to fulfill code requirements in steady state, both discs were open. For diastole, the inlet disc was fully open whereas the outlet one was almost closed. For systole, it was vice versa. The non-Newtonian blood model based on the Power Law was applied in numerical simulations, utilizing ANSYS CFX v.13 code. Streamlines and flow stagnations areas in the blood chamber were analyzed. 46 VADs were collected from cardiac centers after heart support. The VAD blood chamber were divided into sections and evaluated regarding biological deposition detection and analyzing.
Results: The flow stagnation areas estimated numerically have good correlation with localization of fibrin deposition detected in pumps collected after clinical application.
Conclusions: The numerical study shows that steady state simulations are useful in the design of internal VAD geometry, as they need no extreme computational efforts. Clinically collected material confirmed good correlation of numerical analyses, applied for new ventricular assist devices designing in the Artificial Heart Laboratory.

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P42 (EI0344)

DEVELOPMENT OF DURABLE ROTARY BLOOD PUMP SYSTEMS USING HYDRODYNAMIC BEARING TECHNOLOGY

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Objectives: A hydrodynamic bearing system uses the fluid film pressure to keep the non-contacting rotation of the impeller of a rotary pump. This is one of the key technologies to develop a blood pump system with superior durability and antithrombogenicity. Our research team is currently working on the development of two different blood pump sytems with hydrodynamic bearing systems.
Methods: 1. Cardiopulmonary support system with a centrifugal pump: The extracorporeal centrifugal blood pump has the dual hydrodynamic bearing systems in the single impeller. The prototype blood pump system is composed of a disposable centrifugal pump head with the compact driver containing a DC brushless motor. The dimensions of the pump with the driver are 75mm (diameter) x 135mm, weighing 500 grams. The priming volume of the pump is 18mL. 2. Implantable left ventricular assist device (LVAD) with an axial flow pump. The first hydrodynamically levitated axial flow pump was developed in this project. The pump has the dimension of 29mm in diameter and 75mm in length. The weight of the pump is approximately 150 grams. The impeller rotor has a bore in the center, which forms a blood film with the shaft connecting the diffuser and the inlet flow devider. The power consumption of the prototype is about 5 watts at typical working condition as an LVAD.
Results: Both systems were evaluated through a series of chronic animal experiments up to 1 month for the cardiopulmonary support and 3 months for the implantable LVAD. The results of the animal experiments demonstrated a superior blood compatibility of the pumps.
Conclusions: We have succeeded in developing blood pump systems for long-term use applications including a cardiopulmonary support system and an implantable LVAD system.
Acknowledgements: *This research program is supported by the New Energy Development Organization (NEDO) of Japan.*

P43 (EI0288)

DEVELOPMENT OF A MOCK CIRCULATION SYSTEM FOR ENDURANCE TEST OF VENTRICULAR ASSIST DEVICES

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Objectives: We have been developing a mock circulation system for endurance test of ventricular assist devices that can simulate a variety of physiological circulatory conditions. In this study, we compared several inflow and outflow valves for generating pressure and flow waveforms of the systemic circulation on the basis of waveform analysis.
Methods: The mock circulatory loop for endurance test consisted of a pulsatile pump with two closed-chambers (left ventricle and aortic compliance), a control valve (peripheral resistance) and a reservoir (left atrium). Evaluated valves (mechanical prosthetic valves, umbrella and duckbill valves) were mounted in the inlet and outlet of the left ventricle. The pump was driven at 70 bpm, and the aortic pressure (AoP) was set at the 120/80 (100) mmHg. Left ventricular pressure (LVP) and AoP were measured for 1 minute in each valve. Each waveform was evaluated by the spike component extracted with median filter processing, the vibrational component extracted with frequency analysis (FFT) and the slope of each pressure waveform calculated with differential processing.
Results: Examples of obtained evaluation indices were shown as follows: the peak-to-peak of filtered LVP (spike components) and integrated values of power spectrum in spike elements by FFT (vibrational components) were 345.8 mmHg and 518.1 $mmHg^2$ in mechanical valves, 218.8 mmHg and 522.1 $mmHg^2$ in umbrella valves and 162.7 mmHg and 142.4 $mmHg^2$ in duckbill valves, respectively. Thus, the duckbill valve showed the most favorable characteristics in which pressure waveforms were close to the nature systemic circulation.
Conclusions: The endurance test circuit under development was able to generate pressure waveforms with low spike and vibrational components by using duckbill valves. It was suggested that our system has a potential to evaluate various characteristics of prosthetic valves by the simple wave pattern analysis.

P44 (EI0403)

SWITCHING FROM VV TO VA BYPASS IN A PATIENT WITH SEVERE ARDS: INSIGHT FROM A CASE REPORT

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Objectives: To describe clinical consequences while switching from VV to VA bypass in a patient with severe ARDS. To suggest the use of combined VV and VA ECMO support or a VA support with central cannulation in ARDS patients with concomitant cardiac failure.
Methods: A 62-year-old mechanically ventilated patient with hypoxemic respiratory failure due to Influenza A (H1N1) virus, septic shock, multi-organ failure, and unresponsive to rescue therapies, was connected to a VV femoro-femoral ECMO circuit (blood flow 3.4 L/min, gas flow 6 L/min). In spite of an improvement in oxygenation, shock worsened with signs of tissue hypoxia and lactic acidosis. After percutaneous incannulation of the femoral artery the bypass was converted from VV to VA. Following bypass conversion, despite an ECMO blood flow up to 6 L/min, we observed severe desaturation, increase heart rate, decrease arterial blood pressure and cyanosis of the upper body. The patient was therefore connected to a modified extracorporeal circuit consisting of a venous drainage and two reintroductions, one venous (3.4 L/min) and one arterial (2.5 L/min) with two separated ECMO circuits. Vital parameters rapidly improved while arterial lactate decreased and global perfusion ameliorated. To understand the events observed during the switch from VV to VA bypass we tested different combinations of venous and arterial blood flow.
Results: The decrease of VV blood flow while increasing VA blood flow was associated with a severe reduction in mixed venous oxygen saturation and PaO_2, probably caused by a high degree of recirculation of highly saturated blood in the lower body. Cardiac output slightly increased despite increasing arterial ECMO support, likely due to the worsen oxygenation of the upper body.
Conclusions: In a patient with severe respiratory failure and impaired cardiac function, a peripheral femoral VA bypass may not be appropriate to restore adequate physiological parameters.

P45 (EI0076)

LIPOSOME-ENCAPSULATED HEMOGLOBIN REDUCED CEREBRAL INFARCT VOLUME IN RODENT TRANSIENT FOCAL ISCHEMIA

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Objectives: Liposome-encapsulated hemoglobin (LEH, Terumo, Tokyo) is reportedly protective in cerebral ischemia and reperfusion. Search for the optimal oxygen affinity revealed that LEH with high affinity (hLEH, P_{50}=10 mmHg) was more effective than LEH with low affinity (lLEH, P_{50}=40mmHg). In this study, we explored LEH with mid-level affinity (mLEH, P_{50}=17mmHg).
Methods: mLEH (2mL/kg, n=8) or vehicle (saline, n=7) was intravenously infused 5 minutes after thread occlusion of the middle cerebral artery in the rat. The thread was removed after 2 hours for reperfusion and the infarction area was assessed by TTC staining 24 hours later, with periodic neurological function and cortical blood flow monitoring.
Results: While relevant cortical blood flow and neurological function tended to be preserved in mLEH-treated animals, infarct volume in the cortex (98 vs 207 mm^3, $P<0.01$) and striatum (92 vs 164 mm^3, $P<0.01$) and edema formation (106% vs 130%, $P<0.001$) were significantly better suppressed in mLEH-treated rats than in vehicle-receiving control animals.
Conclusions: mLEH reduced edema formation and infarct volume after cerebral ischemia and reperfusion in the rat. This protective effect appears to be no less potent than that of hLEH or lLEH.

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P46 (EI0003)

VARIATION OF LOCAL ELASTICITY ALONG THE LENGTH OF THE AORTA AS OBSERVED BY A SCANNING HAPTIC MICROSCOPE (SHM)

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Objectives: Variations in microscopic elastic structures along the entire length of beagle aorta were evaluated using a scanning haptic microscope (SHM).
Methods and Results: The total aorta from the aortic arch to the abdominal aorta was divided into 6 approximately equal segments. After embedding into agar, each segment was cut in circumferential cross-section to obtain disk-like samples with flat surfaces (thickness, approximately 1mm). The surface elasticity and topography of the samples under non-load and zero-stress conditions were simultaneously measured along essentially the entire wall thickness by SHM, using a probe with a diameter of 5μm and a spatial resolution of 2μm at a rate of 0.3s per point. Elasticity in the wall was the highest at the luminal surface side and decreased gradually toward the adventitial side. The tendency was similar to that of the change in the elastin fiber content. In the evaluation at the mid portion of each segment, the highest elasticity (40.8 [3.5] kPa) was identified at the thoracic section of the aorta that had the highest concentration of elastic fibers.
Conclusions: Under non-load and zero-stress conditions, the elasticity of the aorta was determined by the elastic fiber content.

P47 (EI0169)

WEARABLE ARTIFICIAL KIDNEY: MAINTAINING BODY HOMEOSTASIS THROUGH CONSTANT HEMOFILTRATION

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Objectives: Steady euvolemia in ESRD (end-stage renal disease) patients is almost impossible to maintain with currently available treatment options. Inability to maintain constant dry weight causes discomfort, uncontrollable hypertension and intra- and post- dialytic hypotension that compromise solute removal. We have designed a wearable artificial kidney, an ambulatory ultrafilter that will maintain invariant dry weight and, when used in conjunction with twice-weekly in-clinic dialysis, can provide adequate solute removal.
Methods: The microsieve is constituted of silicon-rich silicon nitride (Si_xN_4, x>3), a hydrophobic non-biocompatible material, which has recently been micro- and nanopatterned to form channels and filtering surfaces that may become valuable components of artificial organs. This material has been rendered hydrophilic and potentially hemocompatible through two different mechanisms: attachment of zwitterionic moieties and deposition of Ti metal followed by oxidation and annealing to TiO_2-anatase. Zwitterionic poly(sulfobetaine acrylamide) (SBMAA) brushes were grafted onto perforated semiconductor microsieves by Atom Transfer Radical Polymerization (ATRP) and, were then, studied in human blood filtration experiments. ATRP initiators were immobilized onto Si_xN_4 through stable Si-C linkages via 3 consecutive reactions. Zwitterionic polymer brushes of SBMAA were grown (thickness ~ 30nm) from these initiator-coated surfaces and the polymer-coated surfaces were characterized in detail by static water contact angle measurements, X-ray photoelectron spectroscopy (XPS), and atomic force microscopy (AFM).
Results: Both the Zwitterionic and Ti-O treatments showed low-contact angles, indicating the surfaces have been made hydrophilic. Furthermore, when exposed to blood on a microfluidic blood plasma separation module (BPSM), a significant decrease was observed in erythrocyte adhesion, platelet aggregation, and protein adsorption. As a result, filtration rate through the microsieve increased by 90-180%.
Conclusions: The device is thus capable of constantly extracting 1 mL/min, about 10kg of ultrafiltrate per week and is designed for inspection and servicing in each dialysis session.

P48 (EI0257)

TISSUE ENGINEERING OF MAGNESIUM STABILIZED, VASCULARIZED, AUTOLOGOUS GASTRIC TISSUE FOR CARDIAC MUSCLE REPLACEMENT

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Objectives: Surgical replacement of diseased cardiac muscle is often the therapy of choice. At this biological patches outmatch synthetic grafts. We showed physiologic *in vivo* remodeling following autologous transplantation of a vascularized segment of small intestine. Nevertheless, the application of such tissue as myocardial patch in the left high-pressure area of the heart is hardly possible due to its mechanical instability. Hence, it was to be examined in this trial if stabilizing of the biological patch by a degradable magnesium scaffold would impair the remodeling and healing process.
Methods: An area of 2cm of diameter in the left ventricular myocardium of Minipigs (n=9) has been replaced by autologous, muscular segments of the stomach including native arterial and venous vessels. This patch was epicardially fixed by a specially designed magnesium scaffold. The grafts were explanted following 1, 3, and 6 months after the surgical procedure and were assessed histologically. Possible enrichment of magnesium and its degradation products was examined chemically. Degradation of the magnesium scaffold was tested with micro-computer tomography.
Results: All animals survived the surgical procedure. Metallic debris was found in the gastric patch and the surrounding myocardium. Increased enrichment of magnesium or its degradation products was not observed in the kidneys, liver, skeletal muscles, and myocardium nor in the bones of the examined pigs. There was no evidence of cytotoxicity of the implanted magnesium. A sterile granulomatous inflammation and a very good capillarization were found up to 6 months after implantation.
Conclusions: The autologous vascularization in our approach is a prerequisite for the *in vivo* remodeling. The use of degradable magnesium scaffolds to stabilize vascularized gastric tissue allows for the application of this initial fragile biological graft in the cardiovascular high pressure area. This trial shows the biological compatibility of degradable magnesium as epicardial scaffolds.

Functional Biomaterials

P49 (EI0414)

A HIGHLY SENSITIVE BIOSENSOR FOR DETECTING TNF-α CYTOKINE TO PREDICT THE BIOCOMPATIBILITY OF TRANSPLANTED ORGANS

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Objectives: Heart failure (HF) is a condition where the heart fails in its duties of circulating blood through the lungs and back out to the tissues. The diagnosis of acute rejection is a complex and persistent problem in heart and ventricular assisted device (VAD) transplantation. To address this problem, measuring specific biomarkers (e.g. TNF-α, IL-1 and IL-10) can produce immediate information about the first signs of inflammation. These biomarkers are usually present within the inflamed organ at high levels and play a major role in coordinating the mechanisms that command the inflammatory response.
Methods: Fabrication of flexible gold microelectrodes based on polyimide for the direct detection of TNF-α required no labeling. Electrochemical impedance spectroscopy (EIS) of the heterostructures, Au/covalently bonded antibodies/buffed medium, was utilized for monitoring the specific antibody-antigen interaction. Experimental parameters affecting antibody immobilization and the sensing of TNF-α were investigated in detail and optimized.
Results: Nyquist plots provide high sensitivity and selectivity for TNF-α versus the antigen IL-1 and IL-10 under optimized experimental conditions. A linear response was obtained in the concentration range 0.02pM to 2pM. The developed biosensor showed a sensitivity of 1,107 M^{-1} and a limit of detection of 0.02pM. Small responses were observed when IL-1 and IL-10 where measured,

showing the selectivity of the biosensor. The reliability and applicability of the developed biosensor was also demonstrated.
Conclusions: The aim of this work was to manufacture a flexible biosensor for detection of TNF-α without any labeling, using electrochemical impedance measurement rather than the traditional techniques. The developed biosensor can be potentially applied for point–of-care applications.

P50 (EI0037)
HEMOADSORPTION OF HIGH-MOBILITY GROUP BOX 1 IN SWINE ACUTE LIVER FAILURE MODEL
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Objectives: We investigated if the adsorption of high-mobility group box 1 (HMGB-1) is feasible and beneficial for the treatment of acute liver failure (ALF) in swine model.
Methods: i) Establishment of animal model. Adult male swine were injected with D-galactosamine (0, 0.2, 0.6, or 1.0 g/kg) to induce ALF. The serum parameters and histological examination in the liver were assessed. Survival was observed for 7 days. ii) *In vitro* absorption study. A multi-cytokine adsorbing column (CYT-860, Toray Inc. Tokyo, Japan) was newly established. The plasma samples containing HMGB-1 were incubated with the fibers of column for 2 hours. iii) Extracorporeal perfusion study. The swine model of 0.6 g/kg was subjected to extracorporeal direct hemoperfusion study. Perfusion was performed for 4 hours using the column, and the HMGB-1 levels at the inlet and outlet of the column were determined. Heptic enzymes were determined at 36 hours after ALF induction. Survival was observed for 7 days.
Results: i) The levels of TB, AST, LDH, and HMGB-1 showed significant elevations in the groups of 0.6 and 1.0 g/kg. Survival study showed that the outcome was dose-dependent. Histological examination of the liver showed hemorrhage and necrosis in the groups of 0.6 and 1.0 g/kg. ii) *In vitro* study showed that the fibers adsorbed 94.3 3.1% of HMGB1. iii) The level of HMGB-1 was markedly suppressed in the outlet compared to the inlet of column during the perfusion with CYT-860. The levels of AST and LDH were markedly suppressed in the group with CYT-860 36 hours after ALF induction ($p=0.06$ in AST, $P<0.05$ in LDH). There was a tendency that the survival was improved in the group with CYT-860 column compared to control column ($p=0.07$).
Conclusions: The newly established cytokine-adsorbing column reduced the serum HMGB-1 level and may be beneficial for ALF treatment.

P52 (EI0325)
MICROPATTERNING OF BIOACTIVE GLASS NANOPARTICLES ON CHITOSAN MEMBRANES FOR SPATIAL CONTROLLED BIOMINERALIZATION
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Objectives: Chitosan membranes were patterned with bioactive glass nanoparticles (BG-NPs) capable of bone regeneration by a Microcontact Printing technique, in order to spatially control biomineralization and also cell adhesion and proliferation.
Methods: After "inking" an elastomeric stamp in BG-NPs, it was pressed against the chitosan substrate and then lifted-off, in order to transfer a perfectly defined bioactive micropattern. The mineralization of the bioactive glass patterns was induced *in vitro* by soaking the samples in simulated body fluid (SBF) over several time points up to 7 days. The interaction between cells and patterned membranes surface was evaluated, by seeding L929 fibroblasts cells over 1, 3 and 7 days on their surface.
Results and Discussion: The induction of confined mineralization was confirmed by FTIR, EDX and SEM. Cell adhesion and proliferation were studied by means of scanning electron microscopy (SEM). The results showed that the produced patterned membranes succeeded in controlling mineralization, cell adhesion and proliferation. MTS assay confirmed that cellular viability increased with time of culture. The developed BG-NPs micropatterned chitosan membranes can be applied in *In situ* tissue regeneration.
Conclusions: The produced membranes proved to be a suitable substrate for cell growth, being the BG-NPs a highly reactive surface able to bond with living cells. Total control of cell attachment and spatial biomineralization was achieved through micropatterning of BG-NPs on chitosan membranes.

P53 (EI0317)
NEW METHOD BASED ON RT-QPCR FOR BIOCOMPATIBILITY TESTING OF DIALYSIS FILTER DEVICES
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Objectives: Objective of this work is to provide a new and more sensitive method based on RT-qPCR (quantitative polymerase chain reaction) for biocompatibility testing in order to facilitate further improvements of dialysis filter devices. Need for improved biocompatibility is given by the clinical observation that dialysis patients have increased risk of cardiovascular disease that is suspected to be influenced by the foreign surface contact. Although there exist a couple of methods to measure biocompatibility, these methods are not sensitive enough to differentiate biocompatibility of modern filter devices and consequently these methods are not suited to guide future developments.
Methods: The method is a two-step *in vitro* process. The first part is the exposure of human blood in parallel to two dialysis filter devices. The second part is the quantitative analysis of the activation level of the leukocyte cell population. The activation level is quantified by the amount of mRNA of a specified set of inflammatory markers by real-time quantitative PCR. Additional information is obtained by FACS analysis of surface marker proteins.
Results: RT-qPCR analysis of inflammatory markers in human blood showed that exposure of blood to different filter devices resulted in different leukocyte activation levels. Particularly early inflammatory markers like TNF-α and IL-1β revealed statistically significant differences between filter devices. Though physical and chemical analysis of the filter materials showed differences in filter materials, no single parameter could be correlated to increased leukocyte activation.
Conclusions: A new method based on RT-qPCR could be established for biocompatibility testing of modern dialysis filter devices. The method allows *in vitro* characterization of inflammatory processes that are caused by foreign surface contact and are suspected to be clinically relevant for cardiovascular complications. Moreover, the method showed differences in leukocyte activation between commercial dialysis filters and is therefore suited to guide further improvements in biocompatibility.

P54 (EI0087)
FUNCTIONALIZED POLYPROPYLENE MESH FOR INCISIONAL HERNIA REGENERATION
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Objectives: The aim of this study was to develop functionalized scaffold for incisional hernia regeneration. New composite scaffolds, based on polypropylene chirurgical mesh (PP), poly-e-caprolactone (PCL) nanofibres, and thrombocyte-rich solution (TRS) have been prepared and tested in *in vitro* study using 3T3 fibroblasts.
Methods: Four different samples have been prepared: PP, PP covered with PCL nanofibres (PP+PCL), as well as PP and PP+PCL functionalized with immobilized thrombocytes (PP+TRS and PP+PCL+TRS, respectively). Nanofibres were prepared by the electrospinning method from the chlorophorm/ethanol solution. To achieve thrombocyte immobilization, PCL nanofibres were immersed in a thrombocyte-rich solution for 2 hours. $1x10^3$ 3T3 fibroblast were seeded onto each scaffold and cultured for 14 days. Cell proliferation and viability were evaluated on the day 1, 3, 7, 10, and 14 by MTT assay and live/dead stainig (BCECF- AM and Propidium iodide) with subsequent confocal microscopy visualization.
Results: Biocompatibility of functionalized surgical mesh, cell proliferation and viability were determined using MTT test and confocal microscopy. The regenerative potential of thrombocytes was based on the release of growth factors that occurs when thrombocytes rupture. MTT test demonstrates significant increase in cell number on scaffold covered with PCL and functionalized with immobilized thrombocytes. These results correlated well with live/dead staining. Viability of cells 14 days after seeding was 95%.

Conclusions: Polypropylene surgical mesh was covered with PCL nanofibre layer, functionalized with immobilized thrombocytes and seeded with 3T3 fibroblasts. Cells proliferated well on the functionalized scaffold during a 14-day experiment. Very good biocompatible properties of this scaffold were observed. This material will be tested and has a good potential to be clinically used.
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P55 (EI0059)
RE-ENDOTHELIALIZATION OF A BIOLOGICAL VASCULARIZED MATRIX (BIOVAM) FOR THE GENERATION OF 3D ARTIFICIAL TISSUES
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Objectives: Tissue engineering is a promising technique for the reconstruction of failing organs. Based on its size the supply of cells with nutrients and oxygen in constructs requires an *in vitro* and/or *in vivo* vascularization, e.g. a biological vascularized matrix (BioVaM). Here we report the generation of a vascularized matrix for 3D artificial tissues.
Methods: An established decellularization process utilizing Na-deoxycholate and SDS was used to generate a matrix with preserved pedicles derived from porcine small intestine. The morphology of the decellularized vessel bed of the matrix was characterized by ink injection and immunostaining. The maximal injection volume of the vessel bed lumen was determined. The matrix was recellularized with lentiviral stable transfected RHE (=rat heart derived endothelial cell line) cells. GFP labeled cells were infused into the venous and RFP labeled cells were infused into the arterial vessel bed. The reseeded matrix was cultivated for 2 weeks under static and/or perfused conditions. After cultivation, the whole construct and cryosections were analyzed via fluorescent microscopy.
Results: Ink injection into the decellularized matrix revealed fine and distinct structures for the arterial vessel bed, whereas the venous vessel bed showed broad and leaky ink distribution. A maximal injection volume was determined. With the adjusted injection volume reendothelialization of arterial and venous vessel bed was achieved after 14 days of static cultivation of the matrix. Perfusion of the matrix was beneficial for the repopulation of big vessel structures.
Conclusions: The arterial vessel bed is well preserved after decellularization; the venous vessel bed is more susceptible to damage due to its native structure of a thin muscular layer. Reendothelialization of the matrix was already achieved under static conditions; however further perfusion might improve functional vessel formation. Perfusable, endothelialized constructs may aid in solving the problem of nourishing cells inside 3D tissue-engineered constructs.

P56 (EI0268)
INNOVATIVE 3D BIOTEXTILES FOR POTENTIAL BONE TISSUE ENGINEERING APPLICATIONS
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Objectives: Bone tissue engineering (TE) represents a specialized niche within the biomedical field to which textile technologies can markedly contribute. Textile technologies are considered as potential routes for the production of scaffolds for TE applications, as they present superior control over design and reproducibility. This work aims at developing novel 3D textile structures based on different polymeric materials and to engineer their surfaces in order to promote and control cell adhesion and proliferation.
Methods: Natural and synthetic polymers such as silk, polybutylene succinate (PBS) and poly(ethylene therephtalate) (PET) were selected to be extruded into multifilament yarns and processed into different structures such as Jersey, Rib and Piqué and 3D spacer. Different surface modifications were performed (acid/alkaline treatment, UV radiation and plasma) for increasing cell adhesion and proliferation. The immobilization of different proteins on the surface of modified materials was also performed. All textile constructs were characterized in terms of porosity, morphology and mechanical properties by μ-CT, SEM and DMA. The effectiveness of the surface modifications was assessed by FTIR, XPS and contact angle measurements.
Results: The obtained constructs present very reproducible intra-architectural scaffold geometry with high surface area and exhibiting a wide range of porosities. By the above-mentioned techniques it was possible to validate the effectiveness of the proposed treatments in modifying the surface of the materials. In addition, Bovine Serum Albumin was successfully immobilized on the obtained surfaces. Cell adhesion and proliferation studies validated the developed constructs for the proposed application.
Conclusions: The proposed textile methodologies made possible the development of highly reproducible constructs featuring a wide range of porosities and surface areas. The effective modification and immobilization of biomolecules on the surface of the biotextiles has the potential for modulating cell response and optimize overall biological performance.

P57 (EI0161)
POLYMER SURFACES COATED WITH HYDROGEL TO IMPROVE BLOOD COMPATIBILITY
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Objectives: The aim of the presented research was to develop a method for manufacturing hemocompatible coatings for blood-contacting devices. We present a simple method for fabrication of hydrogel coatings for cardiovascular devices. Polyvinylpyrrolidone (PVP) was chosen as a hydrophilic polymer to produce hydrogel network due to its highly biocompatibility and wide applications in medicine.
Methods: Hydrogel coatings of polyurethane (in a form of discs) were fabricated in a two-step method. First, the PU discs were immersed in a solution containing given amounts of crosslinking agent (EGDMA) and cumene hydroperoxide for 15 minutes at 25°C. After that time, samples were placed in a water solution containing given amounts of PVP, $FeCl_2$ and ascorbic acid for 15 minutes at 25°C. Polymer discs were then washed and dried. Blood-biomaterial interactions were evaluated using a platelet analyzer (Impact-R, DiaMed). A given volume of a whole-blood sample was dropped onto the characterized surfaces and shear stress was applied to simulate arterial flow conditions. The platelet consumption was calculated as a difference between the initial number of platelets present in blood sample and the number of platelets after the test.
Results: The presented method is based on free-radical macromolecular polymerization. Cumene hydroperoxide is a source of radicals produced in the redox reaction with Fe^{2+} ions. Macroradicals recombination leads to PU-PVP grafting, PVP crosslinking and hydrogel formation. The results showed that the platelet consumption decreased from 56% (for unmodified PU) to 10% (for PU grafted with PVP).
Conclusions: Polyurethane grafted with polyvinylopyrrolidone seems to be a promising material for cardiovascular applications. Hydrogel coating greatly reduced the level of platelet adhesion and activation.

P58 (EI0243)
PERMANENT CENTRAL VENOUS CATHETER WITH A LOCK BALLOON
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Objectives: Permanent vascular access is essential for hemodialysis, parenteral nutrition and drug administration. Mostly a central venous catheter (CVC) is used. However, this use poses a problem: infection. The cause is the intraluminal space, which acts as a bioreactor during the time when the CVC is idle. To prevent this, a bactericidal liquid, called a lock solution, is injected into the intraluminal space. For fluid mechanical reasons, it is not possible to completely fill the intraluminal space without injecting the lock solution into the bloodstream. The proposed lock balloon fills the intraluminal space and makes a lock solution redundant.
Methods: A standard single lumen central venous catheter of 1.4mm inner and 2.1mm outer diameter was chosen to be equipped with a lock balloon. The latter was fabricated from a 25-micrometer polyurethane membrane. The membrane was inserted into the catheter and glued in. A bi-stable actuator was designed and attached to the lock balloon. The lock balloon is filled with 0.3-milliliter air.
Results: During infusion the lock balloon is collapsed and the lumen is free for the passage of the infused liquid. However, the cross section is reduced and the resistance is doubled. After infusion the lock balloon can be inflated again and then it completely fills the intraluminal catheter space. The bi-stable actuator permits to activate and deactivate the lock balloon like a switch.
Conclusions: The concept appears valid and further investigations will deal

with miniaturization of the lock balloon and animal experiments. The animal experiments are designed to model the routine of CVC use. Conventional CVCs will be compared to lock balloon CVCs. Blood cultures after catheter use will be used as a control.

P59 (EI0241)

A CATHETER MODEL FOR THE EVALUATION OF ANTI-BIOFILM AGENTS IN RATS

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Objectives: The colonization of indwelling catheters by bacteria and the formation of biofilms is a frequent problem in today's clinical setting. These infections, mostly caused by coagulase-negative staphylococci, are among the most common nosocomial infections and may even cause the sepsis syndrome. The biofilms are often highly resistant to conventional antibiotics. In this study we evaluate the effect of the antibiotic, e.g. Daptomycin whose therapeutic mechanism is supposed to also affect bacterial biofilms.
Methods: A previously published rat model was modified using a peripheral venous catheter for human use that was adapted for the application in rats. For this study the Teflon tip of the catheter was replaced by a polyurethane tube, to soften and to prolong the line. The system was sterilized using formalin gas and inserted in the right *Vena jugularis externa*. The proximal tip of the catheter was subcutaneously tunneled and passed outwards between the scapulae. To secure the position of the catheter the adjacent plastic wings were sued onto the adjacent tissue. Then the lumen was inoculated with *S. epidermidis* and allowed to indwell within the catheter for 7 days. Afterwards antibiotics were infused in different rats according to their standard instructions for human use. Saline was used as negative control. The rats were sacrificed and the catheters explanted. After embedding in methacrylate and sectioning, fluorescence-in-situ-hybridisation technique (FISH) was used for the quantification of microorganisms and bacterial activity.
Results: The system was well suited for the respective experiments and was well tolerated by the animals. Biofilms were successfully grown in the catheters and could be visualized and quantified using the FISH technique.
Conclusions: This catheter model allows the *in-situ*-analysis of different antibiofilm strategies like antibiotics and the evaluation concerning their biocompatibility and effectiveness in rats.
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P60 (EI0427)

EFFECTS OF PLASMA GLOW DISCARGE ON CHEMISTRY OF PMMA

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Objectives: To examine the effect of plasma parameters on the surface chemistry of PMMA.
Methods: Polymethylmetacrylate (PMMA) is very commonly used for dental applications. In this study PMMA samples were modified by RF oxygen plasma with various powers (10W-50W-100W), which were applied for different periods (5min-15min-30min). The effect of these plasma parameters (power and time) on the newly created surface free radicals, surface chemistry, topography, contact angle and surface free energy were investigated by electron spin resonance spectroscopy (ESR), X-ray photoelectron spectroscopy (XPS), atomic force microscopy (AFM) and goniometer, respectively.
Results: ESR analysis indicated the presence of peroxy radicals on the surface of the oxygen plasma treated PMMA. The intensities of the peroxy radicals increased with increasing plasma power and the application period. The chemistry was also altered. XPS analysis revealed the controlled introduction of functional groups such as carbonate and free carbonyl groups onto the surface of PMMA. Also the roughness of the surface was increased from ~2nm to ~7.5nm after 100W-30min oxygen plasma treatment. The surface-free energy (SFE) was also altered and contact angle results showed that surface wettability can be controlled by changing the plasma parameters. The maximum total dose application (oxygen plasma with 100W for 30min) enhanced the hydrophilicity of PMMA by causing a decrease of water contact angle from 70° to 26°.
Conclusions: Plasma glow discharge can be applied to polymric films to alter their chemical and physical properties.

P61 (EI0096)

3D SMART COMPOSITE COLLAGEN/HA/PCL/PRP SCAFFOLD SEEDED WITH MESENCHYMAL STEM CELLS FOR BONE REGENERATION *IN VIVO*

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Objectives: The aim of this study was to develop suitable composite scaffold for bone regeneration *in vivo*.
Methods: We prepared a Collagen type I (Col)/Hydroxyapatite (HA) scaffold with Polycaprolactone (PCL) nanofibres to improve the mechanical properties of scaffold. The scaffold was seeded with autologous Mesenchymal Stem Cells (MSCs) in osteogenic differentiation media (Group1). We also prepared scaffold (Col/HA/PCL) seeded with MSCs in osteogenic differentiation media enriched with platelet-rich plasma (PRP) as a source of growth factors (Group 2). Both groups of scaffolds were implanted to the rabbit femur condyles where critical size defect 6mm in diameter and 10mm ± 0.5mm in depth was made. Empty defects were used as a control. 12 weeks later rabbits were sacrificed and the femoral condyles were examined by histological analysis. The samples were stained with hematoxylin–eosin (HE), van Gieson's staining, Alcian blue-PAS and Gömöri trichrome staining.
Results: In Group 1 a histological analysis revealed induced production of fibrous tissue which gradually ossificated not only from margin of defects. However, better results were observed on scaffolds enriched with MSCs and with PRP (Group2). There was explicitly predominant direct production of bone trabecules in the whole volume of defects. In empty defects massive blood coagulum were observed with new-formed fibrous scar tissue. Ossifications begin from the margin of defect. From the mechanical testing of scaffolds it was obvious that the moduli of elasticity under compressive test significantly increased at the Col/HA/PCL scaffold compared to Col/HA scaffold without PCL nanofibres.
Conclusions: PCL nanofibres increased mechanical properties of Col/HA scaffold and PRP improved bone regeneration. This smart composite scaffold enriched with PCL nanofibres, MSCs and PRP present new possibilities for bone defect regeneration.
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P62 (EI0237)

TIME-RESOLVED CHARACTERIZATION OF PLATELET DEPOSITION IN A STAGNATION POINT FLOW CHAMBER

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Objectives: Thrombus formation still poses a problem in the development of devices in the cardiovascular system. The process is determined by the components of Virchow's triad, which describes the interaction of blood, flow and surface. The objective of this work is the development of a method to quantify Virchow's triad. For this endeavor the stagnation point flow is chosen.
Methods: For the experiments a stagnation point flow chamber was designed and manufactured. The blood flows through a bore perpendicular onto a flat plate. In a radial distance the blood is collected in an annular channel. This creates a stagnation point flow with axial symmetry but with radially varying shear rates. The flat plate is a microscopic cover slip made out of native glass. It permits a fluorescent video microscopy with an inverted microscope. The blood was drawn from voluntary healthy donors into standard syringes containing sodium citrate. To dye the platelets with calcein red-orange AM, platelet rich plasma is temporarily separated. With a flow rate of 18mL/h the blood enters a microfluidic device to mix with adenosine diphosphate (ADP) (2mL/h, 20 µM) to stimulate the platelets. The activated platelets deposit on the cover slip influenced by shear rate.
Results: In the onset of the experiment the platelets deposit evenly, but shortly after this a preference of certain regions are observed. The region around the stagnation point attracts more platelets, while the stagnation point itself remains nearly platelet free. In addition a development of insular pattern of platelet de-

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position is observed. These insular depositions develop in flow direction with elliptic shapes in the beginning.
Conclusions: With this method temporal resolved platelet formation can be observed using also alternative surfaces modifications. From the results thrombus formation can be modeled considering Virchow's triads.

P63 (EI0132)
EVALUATION OF CYTOTOXICITY *IN VITRO* OF BIODEGRADABLE POLY-LACTIDE FIBERS WITH SPIN FINISHES
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Objectives: Biodegradable fibers with controlled properties may meet the requirements for medical applications. Poly(lactic acid) (PLA) is a biodegradable linear aliphatic thermoplastic polyester. PLA fibers with five-type spin finishes were prepared and assayed for *in vitro* cytotoxic activities.
Methods: The PLA fibers were prepared by a two-step melt-spinning process. The PLA Polymer 6201D, fiber grade with nominal MFI=15-30 g/10min, a NatureWorks LLC product was used. PLA fibers were coated with 5 types of spin finishes: PLA 24 with 2.4% of Glicerol Ph Eur, PLA 25 – 0.40% of Lurol PL 801, PLA 26 – 0.61% of Stantex 6457, PLA 27 – 0.36% of Lurol PT-L216, PLA 28 - 0.62% of Estesol PF 790. The fibers with linear density 2,2 – 4,8 dtex, tenacity 35 – 39 cN/tex, elongation ~50% were obtained. To determine if they can affect cells, line cultures L929 (ATCC CCL1) were used. The cells ($2x10^6$ cells/mL) were incubated with fibers for 24h, 48h and 72h (37°C, 5% CO_2). Cell growth, morphology and viability were determined.
Results: After 72h incubation, the level of cytotoxicity of PLA 24 fibers was 2 (% dead-38), PLA 25 - 3 (% dead-100), PLA 27 - 3 (% dead-100), PLA 28- 0 (% dead-99), control fenol-3 (% dead-94), L929-0 (% dead-3).
Conclusions: Fibroblast cultures after contact with the four of PLA fibers showed cytotoxity effects. The cells were dead with hanged morphologie and lower proliferation. The result of the testing of PLA fibers with Estesol spin finish did not show any cytotoxicity effects and may be a promising candidate for medical applications.
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P64 (EI0213)
CELL COLONIZATION OF POLYURETHANE SURFACE IN THE POLISH EXTRACORPOREAL VENTRICULAR ASSIST DEVICE IN LONG-TERM USAGE
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Objectives: The aim of the investigation was an immunohistochemical analysis of biological material adhered to the inner surface of polyurethane blood chamber of the pulsatile extracorporeal ventricular assist device.
Methods: The investigated materials were pulsatile, extracorporeal VAD, obtained after heart support. The analysis was performed on samples obtained from internal part of VAD's blood chamber. Blood pumps with a working time from 3 to 104 days were examined. An immunohistochemical analysis of the blood contact surface was performed. To detect and differentiate biological material, EnVision method from DAKO and the following monoclonal antibodies against human antigens were used: 1) anti-fibrinogen, 2) anti-actin, anti-CD3(+), anti-CD34, anti-CD45, anti-CD61, and anti-CD68.
Results: The investigated material had an organic character. It consisted of fibrin deposition and several cell types. The cell depositions were well organized forming small cell clusters of erythrocytes, granulocytes, platelets and the low differentiated cells with epithelial and fibroblast morphology. These low organized cells presented mainly monolayer appearance. The highest cellularity of the inner side of the polyurethane wall was observed in cases with long time of VAD working.
Conclusions: Increased time of heart support with the extracorporeal VAD induces the organism into cellularization of artificial surfaces. Several cell populations on different levels of differentiation take part in the construction of a cell monolayer.

P65 (EI0069)
EXPERIMENTAL INVESTIGATION OF MECHANICAL COMPATIBILITY OF A HERNIA MESH
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Objectives: The success of the surgical operation depends in a great extent on the mechanical behavior of synthetic hernia meshes, which are used in the abdominal surgery to repaire different types of hernia. The aim of this work is to evaluate the mechanical compatibility of hernia mesh and human umbilical fascia (UF) comparing their viscoelastic properties.
Methods: Uniaxial stress relaxation tests on mesh specimens – Surgimesh (SM) and fascia samples with dimensions (10x70) mm were performed using testing device FU1000/E. Ten mesh samples were cut along the rows of loops and parallel to the column of loops. Seventeen samples taken from six human cadavers cut parallel to fiber direction and perpendicular to them were used in stress relaxation tests. The initial deformation was 4% and 5% at 1.26 mm/sec rate of elongation. The relaxation process was described by: elastic modulus E, equilibrium modulus E_{eq} when the relaxation process was completed and reduction of the stress $\Delta\sigma$.
Results: Stress reduction for fascia samples varies between 37- 55%, while for SM it was 35-71%. The initial stress for UF was in range 0.11-0.76 MPa and for SM was 0. 34 – 0.59 MPa. The differences between values of the elastic and equilibrium moduli for both materials were not pronounced. The results reveal that the viscoelastic properties of Surgimesh were close to viscoelastic properties of umbilical fascia at chosen levels of strain.
Conclusions: The stress relaxation curves revealed orthotropic and non-linear viscoelastic behavior of investigated materials. Further relaxation tests at higher strain levels are required to determine completely the mechanical compatibility between this brand of hernia mesh and human umbilical fascia.

P66 (EI0026)
BIOMIMETIC MODIFICATION OF SURFACES BY THIOLATED GLYCOSAMINOGLYCANS
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To explore and exploit the bioactivity of glycosaminoglycans (GAGs) towards adhesive proteins, growth factors and cells covalent immobilization on biomaterials or sensor surfaces is required. Three different GAGs (heparin, hyaluronan, chondroitin sulfate) were covalently modified by a disulfide containing crosslinker as a precursor for thiol generation. The thiolated glycans should be used to coat bare gold or vinyl-terminated glass or silicon surfaces to guide the adhesion of cells. The thiolated GAGs were prepared by using a dihydrazide crosslinker that was attached to the carboxylate groups of the glycans backbone. To characterize the immobilization of the thiolated GAGs in terms of quantity and also surface morphology, ellipsometry and atomic force microscopy (AFM) were applied. The wetting properties of the surface after immobilization were studied by water contact angle measurements (WCA) to obtain further information about the degree of surface modification. Human fibroblasts (HF) were used to study the bioactivity of the modified surfaces. The successful immobilization of thiolated GAGs was confirmed via growing layer thickness observed by ellipsometry. AFM measurements also revealed the surface coverage with thiolated GAGs. Furthermore the reduced WCA of the modified substrate indicates the binding of hydrophilic polysaccharides. The cell experiments demonstrated a decrease of cell size and adhesion compared to glass and vinyl-terminated glass surfaces due to the hydrophilic nature of GAGs, which indicate a switch from non-specific to specific adhesion mechanism. The results demonstrate that thiolated GAGs can be effectively immobilized on gold but also vinyl-terminated surfaces, which opens the way of one-step modification of biosensors with different GAG. These sensors can be applied to study the properties of the relevant glycans with their natural binding partners such as different type of proteins and cells.
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P67 (EI0381)

ELECTROSPUN BIODEGRADABLE MATERIALS FOR VASCULAR REGENERATIVE MEDICINE

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Objectives: There is a rising interest for the development of small-sized blood vessels substitutes. Several studies have been focused on the development of a biodegradable graft temporarily able to substitute the blood vessels and allow their complete regeneration after a certain time. We tried to develop a biodegradable material, with optimal mechanical characteristics and the capacity to allow cells adhesion, differentiation and proliferation by electrospinning to obtain a nano-fibrillar scaffold starting from a polymeric solution.
Methods: We report the *in vivo* application on rats of two new electrospun biodegradable materials, specifically designed to create tubular structures. Both biomaterials can be functionalized with several polypeptidic and non-polypeptidic active molecules (growth factors or drugs). In one case PHEA-PLA was co-spun with silk fibroin (Fibro-PHEA-PLA) by a parallel electrospinning process to obtain a scaffold with two different polymeric fibers. In the other case, PHEA-PLA was mixed with polycaprolactone (PCL-PHEA-PLA) to obtain a single spinning solution for the obtainment of hybrid fibers scaffold. The *in vitro* assay showed colonization by fibroblasts in both materials. The scaffolds were implanted in a dorsal fascial pouch on Winstar rats to evaluate their *in vivo* biocompatibility and tissue integration. The scaffolds were removed at 7, 15 and 40 days after implantation.
Results: The pathological findings showed that both materials were totally absorbed after 40 days without any sign of inflammation. A neutrophilic reaction was predominant at 7 days, especially for PCL-PHEA-PLA alone, whereas a lymphocytic invasion was showed at 15. At 15 days Fibro-PHEA-PLA showed a good cell adhesion with a low grade of inflammation. Cell adhesion was confirmed at SEM scan.
Conclusions: This preliminary study showed a good biocompatibility property of the scaffolds that needs of further investigations. The capability of the materials to be functionalized, should allow us to guide the development of bioengineered vessels.

P68 (EI0145)

BIOVAM IN THE RAT MODEL: A NEW APPROACH OF VASCULARIZED 3D TISSUE

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Objectives: A major obstacle in tissue engineering is to create a surgically implantable tissue with long-term viability. Several promising techniques have focused on biological vascularized matrices (BioVaM) with preserved vascular pedicles in the porcine model. However, the handling of this model is time-consuming and expensive. Therefore, our aim was to establish a biological vascularized matrix in the rat.
Methods: Small bowel segments of *Sprague Dawley* rats (250g) were isolated and perfused via cannulation of the superior mesenteric artery and the portal vein. All cellular matrix components were removed by sequential treatment with sodium dodecyl sulfate, sodium deoxycholate, and DNAse. The quality of decellularization was investigated by histology and potential residual DNA by spectrophotometry. Primary endothelial cells (REC) were isolated from the major vessels of *Sprague Dawley* rats. Cells were labelled with fluorescent *cell tracker* and injected into the vascular pedicles of the matrix. Attachment of endothelial cells was assessed using fluorescence microscopy of the whole mount. After one week of culture in a bioreactor, cryosections of the construct were analyzed immunohistochemically.
Results: After decellularization of the matrix, macroscopic and histological examination demonstrated the absence of cellular components with conserved matrix architecture. This was validated by immune-fluorescent DAPI, Laminin as well as HE-stains. Tissue content of DNA was reduced by more than 99%. RECs were characterized by specific staining against eNOS and vWF. After injection into the matrices, RECs attached along the vessel walls, including the capillaries of the intestinal wall.
Conclusions: Rat small bowel segments harvested with intact vascular pedicles and associated vascular network can be successfully decellularized and reendothelialized *ex vivo*. This rat model is an inexpensive and easy-to-handle alternative and appears to be a promising approach for establishing vascularized tissue constructs.

P69 (EI0099)

ADHESION AND GROWTH OF BONE MARROW MESENCHYMAL STEM CELLS ON 3D ORGANIC-INORGANIC COMPOSITE SCAFFOLDS

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Objectives: In this study, we report on the adhesion and growth of bone marrow mesenchymal stem cells on 3D scaffolds, which are fabricated using a novel composite organic-inorganic material by direct laser writing. We investigate the relationship between the scaffold chemistry and topology and cell adhesion and growth. Furthermore, we explore the potential of the fabricated 3D scaffolds in bone tissue engineering.
Methods: The hybrid material comprised a silicon-zirconium inorganic network and pendant organic tertiary amine groups in different mole ratios. The material was prepared using methacryloxypropyl trimethoxysilane (MAPTMS), (2-dimethylamino)ethyl methacrylate and methacrylic acid as the polymerizable monomers, whereas zirconium n-propoxide Zr(OPr)4, and the trimethoxysilane groups of MAPTMS served as the inorganic network forming moieties. 3D scaffolds are fabricated layer-by-layer using direct laser writing of the organic-inorganic composite material, a technique based on multi-photon polymerization. For the *in vitro* study we use early passages (1-4) of bone marrow mesenchymal stem cells isolated from posterior iliac aspirates of donors. We investigate cell adhesion by confocal microscopy and scanning electron microscopy. For the quantification of cell proliferation we use the MTT assay.
Results: Scanning electron microscopy images show a strong initial adhesion of bone marrow MSCs in the first 3 hours after seeding on the structured composite material. We visualize the actin cytoskeleton and vinculin adhesion points of cells grown on the composite material by confocal microscopy. Preliminary proliferation data indicate a significant increase in cell number after 7 and 14 days under conditions with 1% FBS. Our results indicate a novel organic-inorganic composite material, which can be structured into 3D scaffolds and display a high initial cell attachment and promote cell growth.
Conclusions: The strong initial adhesion and proliferation of bone marrow MSCs on the 3D organic-inorganic composite shows a high potential of the material as scaffold for bone tissue repair.

P70 (EI0426)

SURFACE MODIFICATION OF POLYMERIC MATERIALS BY PLASMA GLOW DISCHARGE APPLICATION

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Objectives: The materials used for medical applications can be modified by glow charge plasma in the presence of an active or inert gas or by further processes by binding various molecules covalently to the surface after plasma activation.
Methods: Oxygen plasma glow discharge was applied to different polymers such as polymethylmethacylates (PMMA), polyurethanes (PU), poly L-lactic acids (PLLA) and polylactide-glycolides (PLGA). For PMMA and PLLA, the effect of plasma parameters (power and application time) on the surface chemistry was examined by ESCA, AFM, and surface free energies (SFE) were calculated from the contact angles measured by goniometer. For PU samples, heparin with two different molecular weights was linked covalently after plasma activation and the effects on thrombus formation were detected by incubating the samples with human blood.
Results: It was shown that surface free energy (SFE) affects the cell attchment and for PMMA and PLLA surfaces had the highest cell attachment when the SFE values were about 60 mJ/m^2. It was also shown that combination of antithrombogenic molecules like heparin to the surfaces of PU samples after plasma activation increased their antithrombogenic property. On the other hand, the addition of micro- or nano-sized hydroxyapatite (HAp) particles on surface activated scaffolds made the surfaces more bioactive and osteoconductive,

and enhanced the attachment of osteoblast cells to the modified surfaces.
Conclusions: Plasma glow discharge is a technique commonly used to alter only the surface without affecting the bulk properties of materials. By adjusting the parameters, it is possible to change only the surface chemistry, or to cover the surface with the required molecules.

P71 (EI0326)
ZRO2/PCL HYBRID MATERIAL SYNTHESIZED VIA SOL-GEL: CHARACTERIZATION AND RELEASE KINETICS OF ANTI-INFLAMMATORY DRUGS (anti-inflammatory è un aggettivo, la frase non è complete senza un nome che lo accompagni)
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Objectives: Controlled-localized drug release systems offer several advantages over other delivery options: they may provide the desired constant drug concentrations at the delivery site, lower systemic drug levels, and a reduced potential for deleterious side effects. The aim of this study was to synthesize and characterize novel sol-gel organic-inorganic hybrid materials to be used for drug delivery applications.
Methods: Organic-inorganic hybrid materials of class I based on poly(ε-caprolactone) (PCL 6, 12, 24 and 50wt%) and zirconia were synthesized by a sol-gel method, from a solution containing zirconium propoxide, PCL, water and chloroform. This solution was mixed with a solution of H_2O/Ethanol/anti-inflammatory drugs (Ketoprofen and Indomethacin 5wt% and 10wt%). The release kinetics in a simulated body fluid (SBF) was subsequently investigated and the amount of drug released was detected by UV-VIS spectroscopy. The structure of ZrO_2 gel, PCL and ZrO_2/PCL hybrid materials was investigated by XRD, FTIR and solid-state NMR.
Results: The structure of the hybrids is obtained by means of hydrogen bonds between Zr-OH group in the sol-gel intermediate species and carboxylic group in the repeating units of the polymer, as suggested by FTIR analysis, and strongly supported by solid-state NMR. The ZrO_2 gel and ZrO_2/PCL XRD diffractograms exhibit broad humps characteristic of amorphous materials, while sharp peaks, typical of a crystalline material, can be detected in the diffractogram of PCL. Drugs entrapped in the organic-inorganic hybrids were released with logarithmic time dependence, starting with an initial burst effect followed by a gradual decrease.
Conclusions: The synthesis of amorphous organic-inorganic hybrid materials containing drugs, obtained by sol-gel methods, was performed to devise new strategies for controlled release dosage forms. The release kinetics demonstrates that the investigated materials supply high doses of the anti-inflammatory agents during the first hours and then a slower drug release. The increase in the percentage of drugs increases the speed release.

P72 (EI0391)
HYALURONIC ACID MICROPARTICLES AS INJECTABLE DRUG CARRIER FOR KNEE CARTILAGE REPAIR: EFFECT ON ARTICULAR CHONDROCYTE BEHAVIOR
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Objectives: Cartilage is a specialized connective tissue performing many essential functions in the musculoskeletal system. If left untreated, cartilage injuries can lead to early progression of degenerative osteoarthritis (OA). Ideally, the efficient treatment of OA will require not only therapeutics that will reduce the degenerative processes, but also that will promote regeneration of cartilage. The goal of this study was to produce hyaluronic acid (HA) microparticles as a carrier for the delivery of therapeutic or signaling molecules (e.g. soluble growth factors) that can regulate cell function and cartilage regeneration. We examined the effect of the microparticles on human articular chondrocytes (HAC) viability, proliferation, CD markers and gene expression.
Methods: HA microparticles were produced by water-in-oil emulsion [1] and characterized by scanning electron microscopy. HAC were isolated from femoral condyles by several enzymatic digestions. Cells were cultured in direct and non-direct contact with microparticles, previously sterilized, for 1, 3, 7, 14, 21 and 28 days. MTT and DNA assays were performed to determine cell viability and proliferation. Real time PCR and Flow cytometric analysis (FACS) were performed over time to assess chondrocyte phenotype.
Results: Microparticles with a regular circular shape and having diameters between 8 and 40μm were obtained. *In vitro* culture with HACs showed that microparticles did not show any kind of cytotoxicity and consequent decrease on cell viability. FACS revealed non-significant changes on the expression of CD44, 90 and 105 over time. Expression of COMP, SOX9 and aggrecan (cartilage-specific genes) were upraised when in presence of HA microparticles after 7 and 28 days. Collagen type I and II were fairly constant in both conditions.
Conclusions: Our results suggest that HA microparticles enhance the maintenance of the chondrogenic phenotype over time and can be used as an injectable drug carrier for cartilage regeneration. [1] - Weiliam et al, 2004.

P73 (EI0168)
ANTIFEEDANT EFFECTS OF GAMMA RADIATION AND ROSMARINUS OFFICINALIS TO LARVAL STAGE OF TRIBOLIUM CASTANEUM (COL: TENEBRIONIDAE)
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Objectives: In this study antifeedant effect of combination of gamma radiation and *Rosmarinus officinalis* L. essential oil as a bio-controlling safe method on larvae of flour weevil, *Tribolium castaneum* (Herbst) were studied.
Methods: Doses of 100Gy of gamma radiation and 0.5 and 1 μl/L disk of essential oil were employed, and after 72 hours nutritional indices were evaluated. Relative growth rate (RGR), relative consumption rate (RCR), Efficiency of Conversion of Ingested Food (ECI) and the feeding deterrence index (FDI) as nutritional indices were evaluated. Treatments were assessed by flour wheat disc at 27±1° C and of 65% humidity in dark conditions.
Results: Results showed that relative growth rate had significantly decreased ($P < 0.05$) by combination of gamma radiation and *R. officinalis* and the severity of this reduction when doses increased was higher. Relative food consumption rate also decreased when gamma radiation and *R. officinalis* combined with each other and its value had convert relative with increasing of doses. Experiments showed that the use of gamma ray and *R. officinalis* alone had no significant effect on Efficiency of Conversion of Ingested Food and feeding deterrence effect of larvae and reduction was observed only when they combined.
Conclusions: The results showed that the use of gamma radiation and *R. officinalis* that induces antifeedant effect can be used as an effective method to control *T. castaneum*.

P74 (EI0158)
OIL NANO-ENCAPSULATION BY COACERVATION METHOD ON NUTRITIONAL INDICES OF TRIBOLIUM CASTANEUM (COL: TENEBRIONIDAE)
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The efficiency of nano-encapsulation of *Artemisia sieberi* Besser on nutritional indices of *Tribolium castaneum* Herbst was tested in this study. Several experiments were designed to measure the indices, such as relative growth rate (RGR), relative consumption rate (RCR), efficiency of conversion of ingested food (ECI) and feeding deterrent index (FDI). Treatments were evaluated by the method of flour disk bioassay in the dark, at 27±1°C and 65±5% R.H. Several concentrations were prepared and 10 adult insects were introduced into each treatment. Then, the ingested food and weight gained were measured three days later. Results showed that nano-encapsulation of *A. sieberi* oil was highly effective compared to *A. sieberi* oil, and significantly decreased the RGR and RCR. Moreover the nano-encapsulation of *A. sieberi* oil was more effective on FDI than *A. sieberi* oil.

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Lung Support, valves and varia

P75 (EI0263)

PULMONARY BLOOD FLOW AND PRESSURE AS WELL AS ARTERIAL BLOOD OXYGENATION SIMULATIONS IN VENTILATED ARTIFICIAL PATIENT SUPPORTED BY CONTINUOUS ROTARY BLOOD PUMP

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Objectives: Our previous study suggested insignificant impairment of pulmonary circulation during artificial ventilation if the inspiration time is smaller than the duration of 2-3 heart cycles. This was due to pulmonary blood volume periodic changes. The aim of this study is to analyze the influence of left ventricular assistance by continuous rotary blood pump (RBP) on hemodynamic in ventilated, virtual patient.
Methods: Virtual RBP was added to a previously elaborated hybrid (pneumo-numerical), cardio-respiratory system adapted to simulate left heart failure. Ventilation to perfusion ratio (V/Q) in different lung regions as well as courses of pulmonary resistance (R_p) alteration, pulmonary blood flow (Q_p) and pressure (P_p), alveolar partial pressure of oxygen and arterial blood oxygenation (SaO_2) were analyzed for various parameters of mandatory ventilation and different RBP speeds (various RPM values).
Results and Discussion: Experimental courses illustrate that RBP flow influences V/Q: the greater the P_p value because of low RBP flow, the smaller the influence of hydrostatic pressure on regional R_p. In particular, if the RBP blood flow is low, R_p is smaller because of increased P_p, and thus if the hypoxic vasoconstriction is not present, Q_p through worse ventilated regions (the shunt) is greater causing a decrease in SaO_2. If, however, the hypoxic vasoconstriction exists, results may be different.
Conclusions: An artificial patient makes it possible to analyze complex multi-factor problems, which would be impossible (because of both physical and ethical limitations) in cases of real patient examination.
Acknowledgements: *The research leading to these results has received funding from the European Union Seventh Framework Programme (FP7/2007-2013) under grant agreement no. 248763 (SensorART Project).*

P76 (EI0406)

A NEW PULSATILE MOCK LOOP FOR *IN VITRO* SIMULATION OF HEART VALVE PROCEDURES IN PORCINE HEART

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Objectives: *In vitro* tests can effectively support the development of new surgical techniques or cardiovascular devices for heart valve repair. A mock loop able to house an entire explanted porcine heart and subject it to pulsatile fluid-dynamic conditions was developed, in order to get real-time images of the valvular structures and to allow the performance of simulated surgical procedures.
Methods: The left ventricle of an entire swine heart is connected to an external pulsatile pumping system, consisting in a piston pump which can replicate the systolic and diastolic flow waves. The afterload, connected to the ascending aorta, simulates the human systemic impedance. The preload is achieved through a reservoir, which passively fills the left atrium. Access for endoscopic imaging is in the apex of the left ventricle. The mock loop is equipped with transducers that measure the mitral and aortic flow rates and the pressures in the ventricle, aortic root and left atrium.
Results: The experimental pressure and flow tracings well matched the typical physiological wave shapes. A mean flow of 3.50±0.1 lpm was obtained. The average working pressure of the systemic impedance simulator was 105±13 mmHg, with a good correspondence with the typical physiological values. The pressure drops across the mitral (2.8±0.2 mmHg) and aortic valves (6.7±1.9 mmHg) are coherent with data reported in the literature. Video recordings showed the great potential of the system in the observation of the cardiac structures dynamics in pulsatile conditions.
Conclusions: A mock loop for subjecting explanted porcine hearts to pulsatile fluid-dynamic conditions was designed and tested. The system allows surgeons to simulate heart valve repair procedures or to implant transcatheter valves in a familiar environment and directly analyze their effectiveness. Hemodynamic conditions were consistent with the physiological ones, and real-time videos showing left heart valves dynamics were acquired with endoscopic techniques.

P77 (EI0287)

IMAGE-BASED AUTOMATIC METHOD TO NOT INVASIVELY MONITOR THE HEART RHYTHM OF ZEBRAFISH EMBRYOS

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Objectives: In the last years the zebrafish embryo has been suggested as an ideal model for cardiac research. The overall similarity between zebrafish and human in response to cardiotoxic drugs was demonstrated, for example, in drug-induced cardiac arrhythmia. For this reason, several methods have been developed to assess cardiac rate (CR) in zebrafish embryos. In this study, we present a simple, not invasive method that adopts *1)* confocal microscopy (CM) image recordings of the embryo's heart combined with *2)* image processing, and *3)* spectral analysis to measure the CR of zebrafish embryos.
Methods: Zebrafish embryos at 96 hpf developmental stage were mounted in 0.5% agarose and analyzed with a Leica confocal laser-scanning microscope. Not treated and treated-with-drugs embryos were investigated. Cine CM images visualizing the beating ventricle were captured. A software was developed to automatically estimate heart rate from dynamic images. The software consists of: *1)* detection of the border of the ventricle, obtained applying morphological operations; *2)* calculation, over a selected 2D slice, of the ventricular area and of the blood cells within the chamber at each time frame. Time series were built from sequences of consecutive ventricular area and blood cells number variations during the cardac cycle; *3)* estimation of the CR from spectral analysis of both blood cells number and area variation time histories.
Results: Zebrafish embryos CR was measured not invasively and automatically. Results were validated by experts who reviewed the acquired images by visual inspection. The spectral analysis of the time series extracted from image processing clearly show changes in the CR of the embryos under the effect of drugs.
Conclusions: The not invasive, image-based, fully automated method presented herein allows estimating zebrafish embryo animal model CR avoiding geneticists and biologists to resort to visual inspection or invasive techniques.

P78 (EI0284)

INFLUENCE OF THE MECHANICAL BI-LEAFLET PROSTHETIC VALVE DESIGNS ON THE FLOW FIELD AND ACCUMULATED STRESS INSIDE THE SIMULATED AORTA

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Objectives: Design feature of the bi-leaflet heart valve and existence of Valsalva generate complex flow field inside the aorta and may impose excessive stress on blood component and cause possible platelet activation. Experimental study was conducted to analyze the influence of the heart valve designs and installed orientations on the aortic flow field, turbulent stress distribution and accumulated stress on blood component.
Methods: Three mechanical bi-leaflet prostheses, the St. Jude Medical (SJM), the On-X (OX) valves with straight leaflets and the MIRA valve with curved leaflets were tested inside the simulated aorta. Dynamic PIV system was employed to analyze the aortic flow. Accumulated stress level on a blood component was calculated by tracing a particle turbulent stress level history.
Results: The SJM valve peripheral orifices tend to deflect the flow sideway during accelerating flow phase, while newer designs, the OX and the MIRA valves deflect less. The SJM valve central orifice flow shows slower velocity than newer valves, the OX and the MIRA valves, which show strong flow through all orifices. The OX valve generates simple jet-type flow while the MIRA valve generates strong turbulent flow field due to the curved leaflets. Tracing of stress level on a selected particle through the central orifice showed higher accumulated stress level for the MIRA valve than that of the OX valve.
Conclusions: Newer valves show strong flows through all orifices. The MIRA valve with peripherally curved leaflets generates strong and turbulent flow field and resulted in higher accumulated stress than the OX valve.

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P79 (EI0153)

IMPAIRMENT OF ALVEOLAR GAS EXCHANGE CAUSED BY LAMP OIL ASPIRATION QUANTIFIED IN A PHYSICAL MODEL

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Objectives: Alveolar-capillary gas exchange occurs swiftly through diffusion, e.g. O_2 from the alveolar space to erythrocytes through various "non-negligible" barriers: filmy surfactant layer, alveolar epithelium, interstitium, capillary wall, plasma, and erythrocyte membrane. Serious problems are reported when children drink accidently lamp oil followed immediately by an aspiration or after vomiting resulting in a drastic deterioration of oxygen diffusion causing severe lung complications such as dyspnea. In order to quantify the degree of this adversity, an alveolar model to study the effect of various typical lamp oils as a diffusion barrier depending on its physical properties such as viscosity, density, and surface tension.
Methods: In an "alveolus-model-chamber", the alveolar gas diffusion is mimicked to examine different commercial available types of lamp oils properties, e.g. low kinematic viscosities (<7mm²/s). Perfluorocarbons (FC43), which is a high-density inert fluid, is used as a blood substitute having the same range of oxygen solubility. A thin layer of the chosen oils is spread atop the FC43. The system is continuously flushed by air or pure oxygen. Water and surfactants were used as control fluids. A micro oxygen sensor (UNISENSE®) is placed in the FC43 compartment to monitor continuously the PO_2 variations of different oils.
Results: All types of lamp oils used demonstrate a significant decrease of oxygen diffusion. The amount of impairment depends on the physical properties of the examined oil. The lowest oil resistance amounted to 15 folds in comparison to the resistance of water. The presence of surfactant influenced the degree of resistance to the dynamics of Oxygen uptake.
Conclusions: It is evidently shown that the introduced physical module is able to demonstrate the serious impairment of gas diffusion quantitatively for the investigated lamp oils. It is also important still to quantify the degree of impairment of CO_2 elimination in further model studies.

P80 (EI0233)

A LONG-TERM MONITORING SYSTEM FOR HYPERTENSION PATIENTS – RESULTS FROM ANIMAL TRIALS

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Objectives: Hypertension is one of the most common cardiovascular risk factors; however, hypertension control still remains a great challenge. The purpose of our study was to evaluate a novel monitoring system for recording blood pressure, pulse rate and body temperature of hypertensive patients. The device is designed for minimally invasive implantation into the femoral artery.
Methods: The device was tested in a chronic setting in 12 sheep for a period of three to six months. The implantation of the sensor was accomplished by means of a dedicated sheath (PASIS) in the femoral artery under X-ray-control. A reference sensor positioned contralaterally was used to counter-check the measurement quality and validity of each sensor after implantation. Via regular readout measurements and CTs, the proper functioning as well as the position of the sensor was controlled. Pathologic evaluation was made to investigate the possible ingrowth of the sensor tip and the sensor cable at the end of each trial.
Results: Although the project is still in process, several interesting findings can already be reported. The pressure sensors in general deliver stable pressure history. However, the stiffness of the micro-cable as well as the implantation technique have an important impact on stable positioning of the sensor in the artery and thus on the validity of the pressure curves. Cables with low stability tend to slip out of the artery easily whereas high stiffness may injure the vessel.
Conclusions: The achieved findings are promising and support the feasibilty of a stable long-term blood pressure implant to monitor hypertensive patients. However, there are still some challenges to overcome. Future work will analyze the influence of different cable designs and implantation techniques as well as the validation of ingrowth and explantability.

P81 (EI0217)

PREPARATION OF A COMPLETELY AUTOLOGOUS VALVED CONDUIT WITH THE OPEN FORM OF TRILEAFLETS (TYPE VI BIOVALVE)

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Objectives: In body tissue architecture technology represents a promising approach for the development of living heart valve replacements and preparation of a series of BIOVALVEs. To reduce the degree of regurgitation and increase the orifice ratio, we designed a novel mold for a type VI BIOVALVE. In body tissue architecture technology represents a promising approach for the development of living heart valve replacements and the preparation of a series of biovalves. To reduce the degree of regurgitation and increase the orifice ratio, we designed a novel mold for a type VI biovalve.
Methods and Results: The mold had an outer diameter of 14mm for implantation in beagles, and it was prepared by assembling 2 silicone rods with a small aperture (1mm) between them. One rod had 3 protrusions of the sinus of Valsalva, the other was almost cylindrical. When the molds were embedded in the subcutaneous of beagles for 1 month, the native connective tissues that subsequently developed covered the entire outer surface of the molds and migrated into the aperture between the rods. The mold from both sides of the harvested cylindrical implant was removed, and homogenous well-balanced trileaflets were found to be separately formed in the open form with a small aperture at the 3 commissure parts inside the developed conduit, which had a thick homogenous wall. Exposure of the obtained BIOVALVEs to physiological aortic valve flow in beagles revealed proper opening motion with a wide orifice area. The closure dynamics were suboptimal, probably due to the reduction in the size of the sinus of Valsalva.
Conclusions: We developed a marvelous BIOVALVE with near perfect valve function by greatly altering the design concept of the mold used to prepare the BIOVALVEs. The mechanical behavior of this BIOVALVE might allow its use as a living aortic valve replacement.

P82 (EI0127)

THE INFLUENCE OF THE BICUSPID AORTIC VALVE ON AORTIC ROOT DISEASE. THE PULSED DOPPLER ULTRASOUND AND FLUID-STRUCTURE INTERACTION STUDY

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Objectives: A bicuspid aortic valve (BAV) is the most common congenital heart defect that occurs in adults. The BAV has formed only two leaflets instead of the normal three leaflets of the aortic valve. At the beginning, BAV works correctly. After many years of life numerous complications, such as aortic stenosis regurgitation, aortic insufficiency and infective endocarditis, aortic dissection, and aortic aneurysm are revealed, which require surgical treatment. The knowledge on the pathogenesis and consequences of defects, as a result of hemodynamic changes, is still incomplete. The current question is how much dysfunction of BAV is heritable and to which extent it is caused by hemodynamic changes. Therefore, we studied the influence of the biomechanical properties, the geometry, orientation and methods of correction of BAV on the hemodynamic parameters based on clinical data.
Methods: The segmentation of clinical images (CT), both of the aortic arch with normal Tricuspid Aortic Valve (TAV) and dilated aortic arch with BAV was used to create physical and computer 3D anatomical models. The hemodynamical and mechanical analysis of the complex interaction between the valve leaflets, aortic root, blood flow and blood pressures (flow pattern, turbulence, stagnation area, strain, shear stress, wall deformation) of aortic arch was carried out using both Pulsed Ultrasonic Doppler Velocimeter (PUDV) and Fluid-Structure Interaction (FSI).
Results: The BAV reveals a significant influence on the flow patterns and stresses behind the valves. The Reynolds Normal Stress (RNS) is almost six-fold higher than RNS for normal TAV valves. Aortic wall shear stress reaches a critical value, increasing the probability of aortic wall damage. The BAV also causes the development of asymmetry in the flow, turbulences and vortex behind common coronary leaflet.
Conclusions: The abnormal blood flow patterns caused by BAV seems to be a very important factor in the development of aortic root disease.

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P83 (EI0130)

A PUMPLESS EXTRACORPOREAL LUNG ASSIST FOR PREMATURE NEONATES: THE AACHEN NEONATOX

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Objectives: Gas exchange in premature neonates is regularly impaired by immaturitiy of the lung. But mechanical ventilation, which is vital to sustain oxygenation and CO_2-elimination, causes mechanical and inflammatory destruction of lung tissue. To date extracorporeal oxygenation is no treatment option, i.a. due to the size of available oxygenators and cannulas. We hypothesized that a substantial improvement in gas exchange can be achieved by maintenance of the fetal cardiopulmonary bypass and interposition of a suitable passively driven membrane oxygenator (in the sense of an "artificial placenta").
Methods: From a range of catheters we chose 14 Ga. One-Lumen-Central-Venous-Catheters for cannulation. The ideal insertion depth was investigated on premature lambs [n=6, 2452 g ±1054, 134 days ±2.7 gestational age (term: 150 days)] for maximum flow, resistance and viability. A requirement specification for the complete extracorporeal circuit was based on the collected data.
Results: Based on the first *in vivo*-results a 70mm catheter-length was chosen for the following *in vivo* test series. An oxygenator with 0.09m² gas exchange surface area and 12mL priming volume (19mL incl. tubing) was designed, produced and tested. *In vitro*-tests showed a typical gas exchange of $47mL_{CO2}/L_{blood}$ and $53mL_{O2}/L_{blood}$ at 80mL/min blood flow and 160mL oxygen flow. *In vivo* a mean pCO_2 (pO_2) at the oxygenator inlet of 54±21 mmHg (49±26 mmHg) and at the oxygenator outlet of 34±7 mmHg (160±64 mmHg) at mean blood flow of 91±35 mL/min resp. 33 mL/Kg/min was found. The animals were hemodynamically stable.
Conclusions: In close cooperation between engineers and neonatologists we developed a small oxygenator and extracorporeal circuit which is suitable as a pumpless extracorporeal lung support for premature lambs. We regard this as one step towards the clinical application of the artificial placenta.

P84 (EI0093)

NEW ARTIFICIAL INTERNAL ORGAN TO CONTROL ATRIAL FIBRILLATION

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Objectives: In some previous papers, atrial fibrillation has been reported to be difficult for the control in a lot of cases with conventional therapy. For the development of the new therapeutic device for the atrial fibrillation, implantable cooling device was developed. An implantable cooling device had been consisted from Peltier element with cooling water supply and voltage current controller. Cooling surface would be attached to the surface of atrium.
Methods: The fourth intercostals space had been opened after anesthesia with Halothane inhalation, and various sensors had been inserted; AF was induced by the electrical current with battery.
Results: As the results, AF was recovered to the normal sinus rhythm after cooling with developed devices in all six goats. The method of cooling with implantable device for the control of AF might be evident in these experiments.
Conclusions: Smaller size cooling device has been under development aiming at achieving a totally implantable type. Catheter type cooling device for the insertion by the use of fiberscope type is now under planning for the clinical application. This new type of device may be able to become good news for the patients with uncontrollable AF.

P85 (EI0013)

ARCHITECTURE DESIGN OF A NOVEL SEPARABLE MOLD TO OBTAIN AUTOLOGOUS TISSUE HEART VALVES "BIOVALVES" NON-INVASIVELY

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Objectives: We have developed the autologous tissue heart valved conduit "Biovalves" grown in the recipients' subcutaneous spaces, which were autonomically formed precisely according to the shape of the material molds by encapsulation with connective tissues. In this study, a novel separable mold was developed for non-invasive removing the molds, which were completely impregnated into the formed Biovalve tissues with complex 3-dimentional shape.
Methods and Results: The mold consisted of six main plastic parts. Two were tubular rods (14 or 16mm in diameter), which bound three small hemisphere-shaped parts resembling the 3 protrusions of the sinus of Valsalva. The assembly was fixed with pole by insertion into the hole of the combined two tubes to prepare the molds. The molds were placed into the dorsal subcutaneous pouches of beagle dogs for 4 weeks or goats for 8 weeks. The harvested implants were completely encapsulated with connective membranous tissues. After cutting both ends of the implants the impregnated molds were smoothly removed from each end by separating the molds into the parts without any damage to the tissues, resulting in the acquisition of the flawless Biovalves. The Biovalve conduit had 3 protrusions resembling the sinus of Valsalva. A membranous tissue with the shape of a closed trileaflet valve was formed as intended by its design. *In vitro* functional evaluation and *in vivo* implantation study is ongoing.
Conclusions: A novel separable mold for non-invasive preparation of Biovalve was developed, which is one of the major steps toward its clinical application.

P86 (EI0274)

EARLY ECHOCARDIOGRAPHIC PREDICTORS OF PROGRESSION OF LEFT VENTRICULAR DYSFUNCTION (LVD) IN HEMODIALYSIS (HD) PATIENTS

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Objectives: In the HD course prevalence of LVD increases and its severity enhances. Our aim was to show changes in echocardiographic parameters which could be early signs of development or further deterioration of LVD in HD patients.
Methods: Echocardiography (two-dimensional, pulsed wave, continuous wave, tissue Doppler) was performed in 48 patients (27 men, age 63.6+/-15.1 years, HD vintage 40; 5-154 months) before and after HD session at the beginning and at the end of the 6-month study using Pro-Sound 4000 (Aloka, Japan). Kt/V and laboratory parameters were simultaneously evaluated. Unfavorable differences in echocardiographic parameters, occurring in patients without a change in classification of left ventricular function (LVF) over a study period were assumed to be early predictors of development or deterioration of LVD.
Results: During 6 HD months in 31 patients LVF remained stable, in 12 deteriorated, and in 5 improved. Even in patients with stable LVF, left atrial (LA) diameter (42.0; 36.5-44.0 vs 44.0; 39.0-46.0mm, p=0.003) and LA area (19.3; 16.3-21.9 vs 20.0; 17.0-23.5cm², p=0.013) were greater before HD session, and the HD session induced differences in LA diameter (1.0; 0.0-2.5 vs 3.0; 1.0-4.0mm, p=0.014), LA area (1.30; 0.35-3.22 vs 3.02; 0.69-0.74cm², p=0.028) and right atrial (RA) area (1.01; 0.02-2.38 vs 2.03; 0.70-3.43cm², p=0.040) were greater at the end of the study. Ultrafiltration (2155+/-926 vs 2177+/-952 mL, p=0.666) and inferior vena cava diameter (22.8+/- 2.6 vs 22.7+/-2.9mm, p=0.764) were not different. Differences in LA and RA diameters (among others) were also shown in 12 patients with worsening in classification of LVF.
Conclusions: Increasing atrial diameters under stable hypervolemic conditions before HD session may be the early predictive signs of deterioration of LVF in HD patients. Adequate HD sessions with proper ultrafiltration can compensate these early echocardiographic changes, indicating worse tolerance of increased preload.

P87 (EI0126)

COMPARISON OF THE ADDED COMPLIANCE AND ADDED RESISTANCE METHODS FOR LUNG FUNCTION TESTS

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Objectives: Noninvasive estimation of the total respiratory system compliance (Crs) and resistance (Rrs), which is not easy with present screening methods, could be useful in lung function tests. The purpose of this work was to compare added compliance (AC) and added resistance (AR) methods proposed to measure Crs and Rrs.
Methods: Air is exhaled passively after the maximal inspiration and total respiratory muscle relaxation to AC (AC method) or briefly through AR to the atmosphere (AR method). Mouth pressure changes are analyzed to estimate Crs and Rrs. The AC method: Crs is calculated as AC*P1/(P0-P1) (P0, P1- the maximal and steady state pressures, respectively), whereas Rrs is estimated from the

pressure decay time constant. The AR method: assuming that the mouth pressure is equal to the alveolar one when air does not flow, Rrs is calculated as AR*(P3-P2)/P2 (P2, P3 - the pressures just before and after expiration cessation). As P2/AR is the airflow rate, the AR value does not have to be known, if a flow meter is used.
Results: A suitable measuring system was developed. The methods were tested in the developed measuring system (one by one) and on several patients. Preliminary results showed that Rrs estimation is more trustworthy when the AR method is used (because of troubles with estimation of the time constant) whereas the AC method enables to estimate Crs in more reliable manner. The modification of the measuring system was done to apply the two methods together in turn.
Conclusions: The proposed methods seem to be applicable under clinical conditions as a screening examination to support spirometry interpretation. Rrs estimation in both methods has shown different results. The measuring system and methods with the hybrid platform elaborated by our group can also be useful as a tool for research and educations.

P88 (EI0014)
110 DAYS OF EXTRACORPOREAL MEMBRANE OXYGENATION IN A YOUNG WOMAN WITH POSTPARTAL CEREBRAL VENOUS THROMBOSIS AND ACUTE RESPIRATORY DISTRESS SYNDROME
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Objectives: Extra-Corporeal Membrane Oxygenation (ECMO) is often the last resort for serious Acute Respiratory Distress Syndrome (ARDS) after all noninvasive treatment options have failed to improve the patient's pulmonary condition.
Methods: Here we present the successful long-term therapy with such an ECMO device over 110 days in a 28-year-old woman. She developed a postpartal cerebral venous thrombosis and severe respiratory insufficiency.
Results: Veno-venous ECMO was able to rescue this young patient and can be the ultimate treatment option for patients with severe respiratory failure.
Conclusions: Veno-venous ECMO was able to rescue this young patient and can be the ultimate treatment option for patients with severe respiratory failure.

P89 (EI0140)
HEMODIALYSIS ARTERIOVENOUS FISTULA RELATED COMPLICATIONS IN KIDNEY GRAFT RECIPIENTS
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Objectives: The aim of this historical cohort study was to evaluate data on hemodialysis arteriovenous fistula (AVF) related complications in kidney graft recipients.
Methods: The study cohort included 60 recipients of a kidney transplant with symptomatic AVF complications between January 2006 and April 2011.
Results: From the 60 recipients (mean age 50±10, range 14 to 73 years) 29 (49%) were males. Among all AVF, 45 (72%) AVF were located in the forearm (38 left), 8 (13%) in the upper arm (4 left), and 9 (15%) in the elbow (6 left). Complications occurred in 11.1% (60/538) of kidney graft recipients that were treated in our outpatient transplant unit during the study period. Average duration from renal transplantation to AVF complication occurrence was 46.5 months (range 1 to 209 months). The most common complication was painful thrombosis with or without thrombophlebitis, occurring in 28 patients (46.5%). Other complications were growing aneurysms (28%, 17/60), venous hypertension (7%, 4/60), distal hypoperfusion (7%, 4/60), high-output AVF with cardiac failure (5%, 3/60), trauma (1.5%, 1/60). Three patients (5%, 3/60) experienced problems in the AVF area not related to AVF. A total of 37 surgical interventions were performed in 35 patients (mean age 50±12, range 28 to 73 years). AVF closures were performed in 16/35 (46%). Furthermore, extirpations of aneurysms were performed in 10/35 (28%), extirpation of thrombosed AVF in 1/35 (3%), simple thrombectomies were performed in 5/35 (14%) and thrombectomies with reanastomosis in 2/35 (6%). The majority of surgical interventions were performed by interventional nephrologist, under local anesthesia.
Conclusions: A significant number of kidney graft recipients have complications related to AVF, often requiring surgical intervention. The most common complications are thrombosis, often with thrombophlebitis, and growing aneurysms. Vascular access-related complications after kidney transplantation should be the focus of further studies.

P90 (EI0398)
SEGMENTATION AND GRID GENERATION FOR NUMERICAL SIMULATIONS OF VAD CONNECTIONS WITH PATIENT-SPECIFIC DATA
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Objectives: We are interested in the numerical simulation of the anastomotic region comprised between outflow canula of LVAD and the aorta. Segmentation, geometry reconstruction and grid generation from patient-specific data remain an issue because of the variable quality of DICOM images, in particular CT-scan (e.g. metallic noise of the device, non-aortic contrast phase). We propose a general framework to overcome this problem and create suitable grids for numerical simulations.
Methods: Preliminary treatment of images is performed by reducing the level window and enhancing the contrast of the greyscale image using contrast-limited adaptive histogram equalization. A gradient anisotropic diffusion filter is applied to reduce the noise. Then, watershed segmentation algorithms and mathematical morphology filters allow reconstructing the patient geometry. This is done using the InsightToolKit library (www.itk.org). Finally the Vascular Modeling ToolKit (www.vmtk.org) and gmsh (www.geuz.org/gmsh) are used to create the meshes for the fluid (blood) and structure (arterial wall, outflow canula) and to *a priori* identify the boundary layers. The method is tested on five different patients with left ventricular assistance and who underwent a CT-scan exam.
Results: This method produced good results in four patients. The anastomosis area is recovered and the generated grids are suitable for numerical simulations. In one patient the method failed to produce a good segmentation because of the small dimension of the aortic arch with respect to the image resolution.
Conclusions: The described framework allows the use of data that could not be otherwise segmented by standard automatic segmentation tools. In particular the computational grids that have been generated are suitable for simulations that take into account fluid-structure interactions. Finally the presented method features a good reproducibility and fast application.

P91 (EI0239)
AN AUTO-REGULATION UNIT FOR LEFT VENTRICULAR ASSIST DEVICES
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Objectives: Left Ventricular Assist Devices usually run at a constant blood flow, disregarding the physiological conditions of the patient. The objective of this work is to propose an autonomous regulation unit that varies the pump flow on the fly, depending on several parameters.
Methods: A set of possible physiological signals were considered as input to the auto-regulation unit. In particular, two implantable pressure sensors, placed before the pump inlet and right after the pump outlet are used to measure the current blood pressure and flow. As soon as the pressure at the pump inlet varies, e.g. due to a physiological increase of the oxygenated blood request, the auto-regulation increases the pump speed in order to keep the flow constant. This architecture was preliminarily tested on bench with a Sinergy LVAD (Circulite GmBH) and two custom-made implantable pressure sensor catheters. The pressure sensors were provided by STMicroelectronics. The auto-regulation unit is based on a STM32 microcontroller and runs almost in real time.
Results and Discussion: The auto-regulation unit was able to cope with a decrease in the inlet chamber pressure by adjusting the pump speed instantaneously.
Conclusions: From the preliminary bench testing performed so far, it seems that the auto-regulation of LVAD speed allows a constant flow to be maintained. Next step will be to add other sensors as input to the auto-regulation unit and then try the whole system extensively, first on an animal model, and then on humans.

P92 (EI0166)
MODIFIED OPEN CIRCUIT AND VACUUM-ASSISTED VENOUS RETURN REDUCES BLOOD USAGE DURING CARDIOPULMONARY BYPASS
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Objectives: To determine whether vacuum-assisted venous return has clinical advantages over conventional gravity drainage apart from allowing the use of

smaller cannulas, shorter tubing and reduced priming.
Methods: A total of 80 CABG operations were performed at our institution between July 1999 to December 2010, using vacuum-assisted venous return with small venous cannulas connected to short tubing. These were randomized with 80 CABG operations using conventional gravity drainage. Priming volume, hematocrit value, red blood cell usage, and total blood product usage were compared by means of multivariate analysis.
Results: The priming volume was 780+/-140mL for small-cannula vacuum-assisted venous return, 1300+/-88mL for gravity drainage (P <.0001). Smaller priming resulted in higher hematocrit values both at the beginning of cardiopulmonary bypass (26%+/-5% compared with 21%+/-4%, respectively, P <.0001) and at the end (28%+/-4% compared with 24%+/-4%, respectively, P <.0001). Red cell transfusions were used in 12% of the patients having small-cannula vacuum-assisted venous return and 41% of the patients having gravity drainage (P =.001); total blood product usage was 15% and 61%, respectively (P =.001). Despite a postoperative blood loss, length of stay in intensive care unit was similar in both groups; the association of vacuum-assisted venous return with lowered blood product usage was confirmed also in the postoperative period.
Conclusions: Modified open circuit and vacuum-assisted venous return result in 1) higher hematocrit values during cardiopulmonary bypass and 2) decreased red cell and total blood product usage.

Vascular Access in Hemodialysis

P93 (EI0157)
HYPERTONIC CITRATE CATHETER LOCK CAUSES PROTEIN PRECIPITATION
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Objectives: Between hemodialysis treatments catheters are locked with a locking solution. Because of its antimicrobial properties hypertonic trisodiumcitrate has become popular in Europe. This solution is not only spilled when injected, but is in part exchanged against whole blood due to its high density. Plasma proteins are therefore exposed to hypertonic triodiumcitrate.
Methods: During *in vitro* tests with hypertonic citrate protein precipitation was observed. Subsequent *in vitro* studies showed that this phenomenon occurs at concentrations exceeding 12 percent. When locks were aspirated *in vivo* precipitated protein could be separated and analyzed. The main constituent was albumin. During *in vitro* tests protein precipitation could be observed visually in the lumen of a 2mm tubing, which is equivalent to a catheter lumen.
Results: Hypertonic citrate locks are exchanged against whole blood even up to the highest point in the catheter. During this process plasma proteins come into contact with hypertonic citrate and subsequently precipitate. They may also partially or totally occlude the catheter lumen. Literature search revealed data confirming precipitation of proteins by different salts, which were shown to have consistent effects on the solubility of proteins. “Salting out of plasma proteins by sodiumcitrate” has become a common method for serum protein purification since the 19th century. Nevertheless, none of these papers has considered its relevance with respect to clinical application in hemodialysis patients.
Conclusions: Hypertonic trisodiumcitrate lock solutions are potentially dangerous and may be the underlying cause for reported embolic complications in patients with central venous catheters

P94 (EI0177)
DELIVERED BLOOD FLOW PREDICTS QUALITY OF LIFE IN DIALYSIS PATIENTS
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Objectives: The diminished Quality of life (QoI) plays causative role in adverse outcome in hemodialysis patients. The aim of this study was to elicitate the association between the delivered blood flow and the QoI.
Methods: The SF-36 questionnaire was validated in 121 hemodialysis pts. Data was obtained from history, laboratory findings, and dialysis regime for the previous 3 years. Socio-demographic data: age, gender, socio-economic status, education level, marital status, family support and presence of sleep disturbance. After adjusting for initial failures, the rates of thrombosis of AV fistulas, as number of episodes per patient/year, were calculated. Stenosis and corrections of fistulas were observed. Multivariate regression analysis was performed on QoI scores.
Results: We found that older age, lower social status, sleep disturbance and poor family support were associated with significantly lower QoI scores. Diabetic patients and those living alone scored significantly worse on the Physical and Mental component score. Age (β=-259, p=0.003), family support (β=0.215, p=0.007), sleep disturbance (β=-226, p=0.006), and delivered blood flow (β=261, p=0.003) were the strongest independent predictive markers for the Physical component score. For the Mental component score, family support (β= 189, p= 0.044), sleep disturbance (β=-226, p=0.006), and delivered blood flow (β=273, p=0.003) were the strongest predictors. Analysis of patients with lower blood flows showed significantly higher rates of fistula thromboses (β=-233, p=0.01). Patients with fistula thromboses rates higher than 0.25 episodes per year scored significantly lower for PCS (p=0.042).
Conclusions: The socio-demographic factors are of major impact on the QoI. Lower dialysis blood flow deteriorates the QoI to a remarkable extent, potentially caused by higher rates of fistula thromboses. These data suggest that patients with lower blood flows should be closely evaluated because of higher risk of thromboses of fistulas and impact on overall QoI.

P95 (EI0025)
THE IMPACT ON DIALYSIS ADEQUACY WHEN DIALYZING WITH SINGLE LUMEN OR DYSFUNCTIONAL DOUBLE LUMEN CENTRAL VENOUS CATHETERS
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Objectives: Double lumen (DL) central venous catheters (CVCs) often suffer from thrombosis and/or suction towards the vessel wall, both resulting in non-sufficient blood flow and a negative impact on solute removal during hemodialysis. Reversing the catheter connection often restores blood flows, but might lead to enhanced recirculation. The use of single lumen (SL) CVCs inherently leads to recirculation. We investigated the differences in dialysis adequacy using different settings of CVCs as vascular access, by evaluating total solute removal (TSR) of different solutes.
Methods: A mathematical model was developed, combining a 2-compartmental model (simulating solute kinetics in the patient) and a dialyser model (simulating solute removal in the dialyser). The compartmental model was calibrated based on kinetic studies from literature for urea, phosphate (P) and Beta-2-Microglobulin (β2M). Hemodialysis sessions of 4 hours were simulated in case of well-functioning DL CVCs (blood flow QB350mL/min), malfunctioning DL CVCs (QB250), reversed DL CVCs (QB350 with 15% recirculation) and a 12Fr SL CVC (effective QB273). The TSR, calculated as solute mass in spent dialysate, was calculated for the different catheter settings and the 3 solutes.
Results: For the well-functioning DL CVC, TSR is 787mmol for urea, 43.8mmol for P, and 310mg for β2M. If QB decreases, TSR decreases by 13% (urea), 14% (P), and 18% (β2M), while reversing the catheter connection results in a TSR decrease of only 5%, 4%, and 1%. A 12Fr SL CVC decreases TSR by 14%, 15%, and 18%.
Conclusions: In case of malfunctioning DL CVCs, reversing the catheter connection restoring QB to 350mL/min, does not impair TSR, even not with a recirculation of 15%. Using SL CVCs shows similar TSR results as malfunctioning DL CVCs with QB250mL/min, such that SL CVCs might be an appropriate alternative in some clinical cases.

P96 (EI0373)
IMPACT OF HEMODIALYTIC PROCEDURES AND DIALYTIC DOSES ON ERYTHROCYTE GLUTATHIONE S-TRANFERASE (E-GST) ACTIVITY
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Objectives: Glutathione-S-Transferases (GST) represent a superfamily of ubiquitous enzymes devoted to cell protection and they are thought to play a role in the detoxification of both endogenous and exogenous compounds. Previous study demonstrated an increased e-GST activity in uremic patients. Since hemodialysis is a “detoxification” therapy, we hypothesize that there may be

different e-GST activity levels with different techniques and/or dialytic doses in the uremic population undergoing dialysis. The aims of the study are to compare e-GST activity in normal and uremic subjects, and to correlate the dialytic dose and the hemodialysis technique (convective and diffusive) with e-GST activity.
Methods: e-GST activity was assayed using a new automated procedure. 103 uremic patients divided into two groups based on dialytic procedures; 44 out of 103 patients underwent standard bicarbonate HemoDialysis (HD-group); 59 patients were treated with online-HemoDiaFiltration therapy (HDF-group). 62 MHD patients and 80 healthy subjects (control group) were studied.
Results: Comparing the e-GST activities of the control group (5.6±1.7 U/grHb) versus all uremic patients (9.0±3.1 U/grHb), we observed a significant statistically difference (p<0.0001). Moreover, we observed statistical significant differences for e-GST activity (p=0.0036), Kt/Vurea (p=0.0007) and weekly Kt/Vurea (p=0.0004) in two subgroups of uremic patients. To try to distinguish between dialytic technique and Kt/V as the cause of the different e-GST expression, we divided all 103 hemodialytic patients in two subgroups using 1.3 as cut-off value of Kt/Vurea. In the patients with Kt/Vurea <1.3 (n° pts) e-GST was 9.67±3.23 while in patients with Kt/Vurea ≥1.3 was 8.65± 2.96, without any statistically significant difference (p=0.156).
Conclusions: This preliminary study will be confirmed by a large trial. In fact a very large number of patients need to highlight eGST as a long-term marker of detoxification, such as a "glycate haemoglobin" for the dialysis therapy.

P97 (EI0314)
DAILY SALT INTAKE AND OVERNYDRATION IN PATIENTS ON HEMODIALYSIS
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Objectives: With a progressive loss of diuresis, sodium and fluid restrictions are vital to control the extra cellular volume in patients on hemodialysis (HD). In anuric patients, each 8g NaCl, requires 1L of fluid intake to maintain normal serum sodium. If interdialysis weight gain (IDWG) exceedes 4–4.5% of dry weight (DW), overhydration (OH) can appear. Aim: to investigate the influence of daily salt intake (DSI) on signs of OH in patients on HD.
Methods: In 75 patients on HD, mean age 58.5±9.1 years, the DSI was calculated using the formula: NaCl(g/day)=8*serum Na(mmol/L)/140(mmol/L) [mean weekly IDWG(Kg)*3/6.5]. According to the median level of DSI, they were divided into low and high DSI patients. The total body water (TBW) was calculated using Watsons' formula. Therefore patients were followed for 6 months for: mean weekly IDWG, NaCl 20% given during HD, approaching the DW, hypertension (HT) and night dyspnea. Chest X-ray and heart ultrasound (HU) were performed. Evaluation for daily fluids intake (DFI), habits for salty food intake and habits for salting food with or without probe was made twice.
Results: The median level of DSI was 12.14±2.35 g/day. When age and TBW were dichotomized by mediana, higher DSI were found for age OR0.2:[CI 0.09-0.6], p=0.009, patients with higher levels of TBW OR 6:[CI 2.16–16.75], p=0.0001 and bigger DFI. The amounts of NaCl 20%, approaching the dry weight and collapses insignificantly affected the DSI p=0.09; p=0.169; p=0.151, respectively). Powerful predictive factors for DSI like Na 20% (p=0.0001, ß=0.245), total body water (p=0,0001, ß=0.312) and DFI (p=0.0001, ß=0.207) were found.
Conclusions: Patients with high DSI have a higher risk of having more TBW. Older patients run a high risk of larger DSI. We must be careful with using 20%NaCl and patients must be educated for DFI. Since, low and high DSI patients do not differ in OH signs, we propose investigations about difference in subcutaneous free-of-water storage of sodium in HD patients.

P98 (EI0196)
CONTRIBUTION OF RENAL AND DIALYTIC CLEARANCES TO DIALYSIS ADEQUACY INDICES
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Objectives: The quantification of the respective contributions of continuous residual renal clearance (K_r) and intermittent dialyzer clearance (K_d) to total (renal plus dialytic) indices of dialysis adequacy is currently based on empirical rules. We explored whether a theoretical correct solution to this problem is possible for dialysis adequacy indices (DAI) by expressing them in a uniform way based on the assessment of the amount of solute (M_R) removed per one dialysis cycle (T_c = one week).
Methods: Two types of DAI can be defined: a) equivalent continuous clearance (ECC) and b) fractional solute removal (FSR) defined as M_R divided per: 1) reference solute concentration in blood and T_c, and 2) reference solute mass in the body, respectively, with the "reference" meaning either: 1) peak, *p*, 2) peak average, *pa*, 3) time average, *ta*, or 4) treatment time average, *trta*, values (Waniewski et al, 2010). Computer simulations were carried out for urea and creatinine applying a variable volume two-compartment model of conventional, daily and nocturnal hemodialysis with constant solute generation rate, and K_r between 0 and 10 mL/min.
Results: Using the definitions of FSR, ECC, K_d and K_r, one can derive the following formulas: $ECC_{ref}=g_{d,ref}\cdot(T/T_c)\cdot K_d+g_{r,ref}\cdot K_r$ and $FSR_{ref}=g_{d,ref}\cdot K_d\cdot T/V_{ref}+g_{r,ref}\cdot K_r\cdot T_c/V_{ref}$ (where T is treatment time, $g_{d,ref}=C_{trta}/C_{ref}$, $g_{r,ref}=C_{ta}/C_{ref}$, V_{ref} is reference volume, and ref=*p*, *pa*, *ta* or *trta*). The weighing coefficients $g_{d,ref}$ and $g_{r,ref}$ were estimated using computer simulations. The coefficients $g_{r,ref}$ and $g_{d,ref}$ cannot be both equal for the same reference method. The calculated values of these coefficients were between 0.32 and 1.46.
Conclusions: Renal and dialytic contributions to DAI can be added by applying weighing coefficients that depend on the dialysis schedule and reference method, but *not* on the type of DAI (ECC or FSR). These coefficients can be calculated using the reference concentrations during the dialysis cycle.

P99 (EI0120)
SURVIVAL OF HEMODIALYSIS PATIENTS WITH CHRONIC HEPATITIS C VIRUS INFECTION
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Objectives: The impact of hepatitis C virus (HCV) infection on mortality of hemodialysis patients remains controversial. The aim of the study was to estimate the survival of HCV positive and HCV negative hemodialysis (HD) patients.
Methods: We conducted a prospective study with 149 HD patients followed from 01 January 2003 till 31 December 2010. Patients' survival was estimated by the Kaplan-Meier method and compared by the log rank test. The Cox proportional hazards model was used to estimate the risk of death in HD patients with HCV infection.
Results: Of the 149 patients, 80 (53.7%) patients were men. Mean age of patients was 59.3±13.8 years with mean dialysis duration of 34.1±23.5 months. Hepatitis C virus infection was presented in 26.2% (39/149) of the HD patients. HCV positive patients were characterized with significantly longer dialysis duration compared to HCV negative, (48.9±21.6 *vs.* 28.9±21.96 months, p=0.000). During the study, 50 (33.6%) patients died. Cardiovascular disease was the main cause of death in 64% (32/50) of the dialysis patients. Patient survival was 56.4% (22/39) for HCV positive and 70% (77/110) for HCV negative patients. Log rank statistics showed no significant difference in survival between HCV positive and negative patients. (log rank, p=0.150). By Cox regression analysis, after adjusting for age, gender, and dialysis duration, HCV positive patients were characterized with a 0.43-fold higher chance to die compared to those without HCV infection (HR = 0.43, 95% CI 0.22- 0.83, p=0.011)
Conclusions: There was no significant difference between HCV positive and HCV negative patients in terms of survival, but HCV infection remains a relative risk for mortality in hemodialysis patients.

P100 (EI0251)
ATHEROSCLEROSIS AND ITS RISK FACTORS AS PREDICTORS OF FEMORAL NECK (FN) BONE MINERAL DENSITY (BMD) IN HEMODIALYSIS (HD) PATIENTS
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Objectives: There are some reports that atherosclerosis and its risk factors are associated with BMD. The aim of our study was to assess the possible association between atherosclerosis, its risk factors and FN BMD and bone metabolism seromarkers in HD patients.
Methods: The study was performed in 60 HD patients (26 women, age 54.8±15.3 years, dialysis vintage 36.9, 6.1-279.6 months). BMD was measured in the FN. Blood pressure, lipid profile and blood levels of homocysteine (Hcy), lipoprotein(a), phosphorus and bone metabolism seromarkers were evaluated.
Results: HD patients with symptomatic atherosclerosis disclosed, after adjustment to age and waist circumference, lower T score (p=0.010), BMD as % of young adults (p=0.011), Z score (p=0.027), BMD as % of age matched (p=0.018). Next to age, presence of symptomatic atherosclerosis and total cholesterol (corr. R^2=0.695) or LDL-cholesterol (corr. R^2=0.680) were negative FN BMD predictors. The correlations between some bone metabolism seromarkers and atherosclerosis risk factors were positive [total alkaline phosphatase

(ALP) with Hcy; tartrate-resistant acid phosphatase 5b (TRAP5b) with Hcy and phosphorus; C-terminal cross-linking telopeptide of type I collagen (CTx) with Hcy, phosphorus, diastolic and systolic blood pressures; osteocalcin (OC) with Hcy, phosphorus and diastolic blood pressure; osteoprotegerin (OPG) with phosphorus], of which ALP and OPG were negatively related to BMD. In the regression analysis, positive predictors for bone metabolism seromarkers, being negatively related to FN BMD, were found among atherosclerosis risk factors: for intact parathyroid hormone (corr. R^2=0.731)- phosphorus, triglycerides (TG); for ALP (corr. R^2=0.889)- Hcy, diastolic blood pressure; for TRAP5b (corr. R^2=0.356)- Hcy, phosphorus; for CTx (corr. R^2=0.472)- Hcy, phosphorus, TG; for OC (corr. R^2=0.464)- Hcy, phosphorus, diastolic blood pressure; for OPG ligand (corr. R^2=0.894)- Hcy.
Conclusions: Symptomatic atherosclerosis and its risk factors by positive relation with some bone metabolism seromarkers adversely influence FN BMD.

P101 (EI0162)
DO SODIUM LOAD AND FLUID INTAKE PREDICT QUALITY OF LIFE IN HEMODIALYSIS PATIENTS?
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Objectives: The diminished Quality of life (QoI) plays a causative role in adverse outcome in hemodialysis patients. The aim of this study was to elicitate the most powerful predictors of the patients QoI among the sodium load and fluid intake.
Methods: The SF-36 (Sort Form – 36 Health Survey) that includes 8 different dimensions of health and two summary scores Physical Component Score (PCS) and Mental Component Score (MCS) were validated in 75 hemodialysis pts. Patients with decompensate heart failure were excluded. Sodium load was evaluated from daily salt intake (calculated using the formula NaCl (g/day) = 8*serum Na (mmol/L)/140(mmol/L)(mean weekly interdialysis weight gain (IDWG) (Kg)*3/6,5)), mean weekly amounts of NaCl 20% given during HD, habit for salty food intake (1.5g sodium in 100mg food/number of portions per day) and adding salt in food.
Results: In the univariate analyses patients with habit for adding salt in food, collapses during HD and absence of edema had lover scores for mental QoI. Habit for adding salt in food, collapses during HD and absence of edema remained most powerful predictors of the mental QoI. In the univariate analyses for physical QoI, patients with habit for adding salt in food, salty food intake and absence of oedema had lower scores for mental QoI. All these factors remained most powerful predictors for physical QoI (β= -0.230, p=0.094). Habit for adding salt in food, collapses during HD and absence of edema remained most powerful predictors of the mental QoI.
Conclusions: The habit for adding salt in food, salty food intake and collapses during HD are associated with lower mental and physical QoI. The presence of oedema in patients with higher QoI is explained with better nutritional status. Other clinical signs for overhydration do not predict the QoI in hemodialysis pts.

P102 (EI0282)
EFFICIENCY OF HIGH CUT-OFF MEMBRANE HEMODIAFILTRATION IN MYOGLOBINURIC RENAL FAILURE
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Objectives: The treatment of myoglobinuric acute renal failure by convective dialysis techniques allows a higher removal of myoglobin (20 kD) than achieved by standard dialysis. High cut-off (HCO) dialyser membranes developed in the last years allow the passage of molecules up to 60 kD molecular weight. We evaluated the removal of myoglobin by hemodiafiltration with the use of the HCO membrane.
Methods: Nine patients were treated by 14 hemodiafiltration procedures with high cut-off membrane (HCO HDF) for severe myoglobinuric acute renal failure. Rhabdomyolysis was caused by infection in two patients, and by a nontraumatic crush in three patients, it was statin-induced in two, and followed cardiovascular surgery in two patients. HCO HDF were performed with a high cut-off hemofilter at dialysate flow 500mL/min, and blood flow within 250-300mL/min, with citrate anticoagulation and postdilutional fluid substitution of 2-3 L/h. Albumin losses were replaced by infusion of human albumin solution.
Results: Pre-procedure blood myoglobin concentration averaged 81mg/L (range, 7.3-223.9mg/L, mean, 58.2mg/L). Serum myoglobin decreased exponentially during the procedure with an approximate half-time of 1h. The myoglobin reduction ratio was 79.4% (range, 76% to 89%). An excellent clearance of 92mL/min (range, 42-131mL/min) was achieved. The highest cumulative amount observed of myoglobin removed into dialysate in the course of the procedures was almost 5 grams. Three of the patients died. Four of the survivors remained anuric for a period of 1-4 weeks, and mostly regained their renal function afterwards. The remaining patients begun to pass urine already at the end of the procedure.
Conclusions: Highly efficient myoglobin removal by high cut-off membrane hemodiafiltration was demonstrated in our patients.

P103 (EI0101)
A POTENTIAL USE OF POLYCLONAL FREE LIGHT CHAIN LEVELS FOR MONITORING IN A CHRONIC DIALYSIS POPULATION
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Objectives: Polyclonal free light chains (FLCs) are potentially an ideal marker for middle molecular weight uremic toxins. This study investigated whether FLC levels were influenced by treatment variables and residual renal function (RRF) in chronic haemodialysis populations.
Methods: Polyclonal FLC concentrations were measured pre- and post-dialysis from two international centres. FLC concentrations were compared between different treatment modalities and RRF (>500mls per 24hours). Patients were excluded from analyses if they had experienced infection or significant illness within the previous 3 months.
Results: Of the 112 patients recruited, sixty patients were anuric and 52 had RRF. The anuric patients had significantly increased total FLC levels compared with patients who maintained RRF (table). Patients between the two centres were comparable in terms of age, sex and vascular access, although patients at centre1 had higher CRP and FLC concentrations (table) than centre2 [CRP: (7.55mg/L (0.12-170)] and 0.7mg/L 90.3-6.6 respectively, p<0.001 for both). Serum CRP concentrations did not correlate with the FLC levels, and no difference in CRP was observed between anuric and RRF patients. The use of HDF significantly increased the FLC percentage reduction from 39% (14-70) to 61% (43-81, p<0.001). The vascular access (AVF or central venous catheter) did not affect the FLC concentrations [326.5mg/L (70.95-738.5) and 280.7 (18.76-687.4) respectively].
Conclusions: Polyclonal FLC concentrations are influenced by both residual renal function and dialysis modality. The measurement of FLCs could act as a simple marker for monitoring concentrations of difficult to remove middle molecule weight uremic toxins.

P104 (EI0046)
INTERLEUKIN-18 (IL-18) PROMOTER POLYMORPHISM IN RELATION TO GENDER OF PATIENTS TREATED WITH INTERMITTENT HEMODIALYSIS (HD)
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Objectives: Serum concentration of IL-18 is increased in HD patients. The *IL-18* -1297C>T (rs360719) polymorphism may modulate the *IL-18* expression. The CC homozygote in IL-18 promoter increases IL-18 transcription. Our aim was to check differences in the *IL-18* -1297C>T polymorphism between HD patients and healthy controls, which could be at least a partial explanation for increased serum IL-18 in HD population. The potential influence of gender on the *IL-18* -1297C>T polymorphism was also analyzed.
Methods: The frequency of *IL18* -1297C>T genotypes was identified by polymerase chain reaction-restriction fragment length polymorphism in 438 HD patients (190 women, 248 men) and compared to 421 (324 women, 97 men) controls.
Results: The frequencies of -1297CC, -1297CT and -1297 TT genotypes were 4.8%, 44.0% and 47.9% in HD patients and 6.2%, 42.0% and 51.8% in controls. The p value for CC vs. CT + TT was 0.459, for CC + CT vs. TT was 0.905, and CC vs. TT was 0.530. The frequencies of -1297CC, -1297CT and -1297 TT genotypes were 5.3%, 42.1% and 52.6% in HD women and 5.2%, 40.4% and 54.3% in healthy women. The p value for CC vs. CT + TT was 0.994, for CC + CT vs. TT was 0.780, and CC vs. TT was 0.938. The frequencies of -1297CC, -1297CT and -1297 TT genotypes were 4.4%, 45.6% and 50.0% in HD men and 9.3%, 47.4% and 43.3% in healthy men. The p value for CC vs. CT + TT was 0.140, for CC + CT vs. TT was 0.317, and CC vs. TT was 0.110.
Conclusions: The *IL-18* -1297C>T polymorphism is not gender-dependent.

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There is no difference in the *IL-18* -1297C>T polymorphism between HD and healthy subjects. Thus, differences in the *IL-18* -1297C>T polymorphism between HD and controls do not contribute to increased serum IL-18 in HD patients.

P105 (EI0052)

HEALTH-RELATED QUALITY OF LIFE IN HEMODIALYSIS PATIENTS WITH DIFFERENT TYPE OF ARTERIAL CALCIFICATION FINDING

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Objectives: There is a lack of studies investigated health-related quality of life (HRQoL) in hemodialysis (HD) patients depended of the type of the arterial calcification (AC) finding. The aim of this study was to evaluate whether the presence of the different type of AC may impact HRQoL in HD patients.
Methods: In a cross-sectional study we examined 88 HD patients (52 men; mean age 54.2±11.8 years; HD duration 121.6±72.4 months). Primarily, we evaluated the presence of arterial intima (AIC) and arterial media calcifications (AMC) using plain radiography of the pelvis. The scales for mental component summary (MCS) and physical component summary (PCS) were derived from eight different subscales originally developed for the short form health survey (SF-36). We compared PCS and MCS scores among the groups of patients with different type of AC (group without AC, group with AIC and group with AMC) presence on radiograms.
Results: Patients (n=33) with AMC finding on radiograms had lower (p=0.008) PCS (42.58±26.39 vs 55.83±27.43), as well as lower (p=0.018) MCS (44.76±24.83 vs 52.66±24.49) score in comparison with the group of patients (n=25) with absence of AC. On the other hand, patients with the AIC (n=30) finding on radiograms had higher (p=0.041) only PCS (47.88±26.22 vs 41.77±28.54) score in comparison with group of patients without AC. We did not find any difference in both PCS and MCS scores among the group of patients with presence of AIC and AMC. The groups did not differ significantly in variables that may affect the HRQoL of HD patients, such as age, gender, hemoglobin, serum albumin and dialysis doses.
Conclusions: HD patients with AC finding on plain radiograms of the pelvis had lower HRQoL scores. This implies that clinical investigations aimed at preventing appearance of the AC in HD patients are still needed to improve patients' quality of life.

P106 (EI0049)

K/DOQI GUIDELINES ATTAINMENT OF BONE AND MINERAL METABOLISM MARKERS AND RELATIONSHIP WITH HEALTH-RELATED QUALITY OF LIFE IN HEMODIALYSIS PATIENTS

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Objectives: The aim of this study was to evaluate whether K/DOQI guidelines achievement of the serum mineral and bone disorder (MBD) markers may impact health-related quality of life (HRQoL) in hemodialysis (HD) patients.
Methods: In a cross-sectional study we examined 82 patients (51 male; mean age 54.5±23.9 years) dialyzed on average for 118.7±61.3 months. The scales for mental component summary (MCS) and physical component summary (PCS) were derived from eight different subscales originally developed for the short form health survey (SF-36) questionnaire. The proportion of the MBD K/DOQI guidelines achieved markers taken from the last 12 months measurements were compared among the groups of patients with various PCS (cut off = 43) and MCS (cut off = 51) levels.
Results: Patients (n=42) with PCS > 43 had significantly higher percentages of attained K/DOQI recommended levels for corrected serum calcium (Ca) (311/414, 75.1% vs 156/397, 39.3%), serum phosphate (P) (352/415, 84.8% vs 191/402, 47.5%) and serum intact parathyroid hormone (iPTH) (33/71, 46.5% vs 12/63, 19.1%) in comparison with the patients with PCS <43. On the other hand, patients (n=44) with MCS >51 had significantly higher percentages of data attainment for serum P (364/439, 82.9% vs 179/378, 47.4%) and iPTH (32/75, 42.7% vs 13/59, 22.0%) in comparison with the group of patients with MCS <51. There was no difference in the attainment of the recommended levels for corrected serum Ca among the group of patients divided according to the MCS. The groups did not differ significantly in Ca x P levels, as well as in variables that may affect the HRQoL of HD patients, such as age, gender, hemoglobin, serum albumin and dialysis doses.
Conclusions: Our evidence shows that greater HRQoL could be accomplished if a higher proportion of the K/DOQI recommended serum MBD levels is achieved.

P107 (EI0040)

HOW TO MEASURE THE DOSE OF DIALYSIS? FORMAL UREA KINETICS VERSUS APPROXIMATION FORMULAE

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Objectives: Kt/V is generally accepted as a parameter which describes the individual dose of dialysis as the product of urea clearance (K) and duration of a treatment (t) related to the urea distribution volume (V) of a patient. Dialysis dose Kt/V can be determined either using a formal urea kinetic model or approximation formulae derived from formal urea kinetic model.
Methods: Data were processed from pre-, post- urea concentrations in a group of 102 patients dialyzed in Dialysis Centers in Aachen and Remscheid/Germany. Kt/V was calculated using the formal urea kinetic model of Stiller&Mann and the second generation Daugirdas (II) approximation formula. The Stiller/Mann model is calculated as a two-compartment model in two versions. The first one considers V estimated by the Watson formula (*St&M I*). The second one includes residual renal function, frequency of dialysis and urea distribution volume measured by bioimpedance (*St&M II)* (BodyScout® Fresenius Kabi GmbH, Bad Homburg, Germany). Residual renal function was measured as the daily amount of urine.
Results: The differences between Kt/V values of *Daugirdas (II)* and *St&M II* are about 29-31% considering frequency of dialyses per week, 7.8% with residual renal function more than 1000 mL/day, and 2% and 3.17% in patients with body fat less than 13% and more than 35%, respectively.
Conclusions: The approximation formulae are only applicable in three times dialysis therapy per week, residual diuresis less than 1000mL and body fat between >13% and <35%. Since it is important for an individual patient to obtain an individual amount of artificial kidney therapy, in all cases where individual data deviate considerably from normal a formal urea kinetic model should be applied.

P108 (EI0023)

HOW ADEQUATE IS MID DILUTION HDF IN THE REMOVAL OF PROTEIN BOUND SOLUTES?

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Objectives: Convective dialysis strategies either in pre- or post-dilution have been proven superior dialysis to remove protein bound solutes (PBS). The concept of mid dilution, combining pre- and post-dilution, might be a promising alternative to the more classical concepts. Therefore, we compared the removal of PBS between post- and mid-dilution hemodiafiltration (HDF).
Methods: The present cross-over study included 14 stable hemodialysis (HD) patients. They were kept for 4 weeks on high-flux HD with one session on either post- or reversed mid-dilution HDF during the midweek session of weeks 3 and 4, performed in random order. Blood and dialysate flows were 300 and 800mL/min in both modalities, while dilution flow was 75mL/min in post- and 150mL/min in mid-dilution. During the test sessions, partial collection of spent dialysate was done to calculate Total Solute Removal (TSR). Blood was sampled at the inlet and outlet blood line to calculate the Extraction Ratio (ER), and pre- and post-dialysis to calculate Reduction Ratio (RR) of PBS and the small water-soluble solutes urea, creatinine, and uric acid.
Results: For the PBS, no differences were observed for the TSR, ER, and RR. Minor but significant higher ER was found with post-dilution for urea (0.92±0.02 vs 0.87±0.04 - P<0.001), creatinine (0.92±0.02 vs 0.88±0.02 - P<0.001), and uric acid (0.84±0.02 vs 0.82±0.03 - P=0.009). The combination, however, of this higher ER with a faster decrease in blood inlet concentration with post-dilution HDF, resulted in a TSR and RR which were not different from those with mid-dilution HDF.
Conclusions: Since TSR was not found different in this cross-over study, post- and mid-dilution HDF were found just as adequate for the removal of small water-soluble solutes as well as of PBS.

P109 (EI0015)

SODIUM PROFILED HFR FOR THE TREATMENT OF DRUG-RESISTENT HYPERTENSION IN RDT PATIENTS

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Objectives: Arterial hypertension is a common complication in uremic patients; although the sodium and water balance should be corrected by dialysis treat-

ment, many patients on regular dialysis treatment still need anti-hypertensive medications. The basic assumption of the present study was to asses whether an improved sodium balance could lead to better control of blood pressure.
Methods: Two male patients on RDT were severely hypertensive despite multiple therapy and appropriate dry weight as assessed by impedancemetry. They were treated for 9 months with HFR Aequilibrium® (Bellco, Italy) (sodium and ultrafiltration profile) increasing the net amount of sodium removed per session without modifying the length of dialysis.
Results: The first patient presented a slight reduction in blood pressure without changes in the antihypertensive therapy; the second patient discontinued antihypertensive medications to normalize blood pressure after 8 months from the beginning of the study.
Conclusions: The sensor for sodium is useful in evaluating the amount removed during dialysis treatment and may help improve treatment outcomes.

P110 (EI0007)
NON-ADHERENCE TO MEDICAL INVESTIGATIONS IMPACTS MORTALITY OF DIALYSIS PATIENTS
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Objectives: Compliance depends on gender, race, occupation, education, income. Missed/shortened dialysis sessions, adherence to therapy, excessive phosphate levels and interdialyitic weight gains (IDWG) and smoking usually provide non-compliance indicators. Aims: to investigate non-adherence to medical investigations as non-compliance indicator and the impact on outcome of patients.
Methods: 126 dialysis patients were scored for indicators of compliance from 0-2 and summary scores of were assessed. Patients with mean IDWGs >4.5% of dryBW and/or phosphorous level above 1.6mmol/L were scored with 1, IDWG/BW more than 5.7% and/or 2.0 for phosphorous with 2. Patients were followed for 24 months up until death, transplantation or end of observational period. Compliance scores of survived and deceased patients were compared with Mann-Whitney test.
Results: Estimated rates of noncompliance: medical investigations 63%, phosphorous 33%, IDWG/BW 22%, therapy 14%, treatment 9%. Noncompliance rate was 73%, adding adherence to medical investigations the rate rose up to 87%. We found the younger age (β=-0.294, p=0.01), lower family support (β=-0.294, p=0.01), lower education (β=- 0.200, p=0.025), smoking (β=0.265, p=0.003) and lower socio-economic level (β= 0.365, p=0.0001) with diminishing effect on the score. In the multivariate analysis, the younger age (β=-0.230, p=0.008) was the most powerfull predictor of non-compliance. The compliance for medical investigations was predicted by lower income (β=-0.221, p=0.018) and family support (β=-0.274, p=0.008). After 24 months 23 (19%) patients died. Non-compliance scores were higher in the deceased population: medical investigations (p=0.006), therapy (p=0.022), dialysis regime (p=0.029). The score for the dietary fluid, medications and treatment regimen was not found to be different between survived and deceased (p=0.129), but when adherence for investigations was added the difference became significant (p=0.047).
Conclusions: The non-adherence to medical investigations has a major impact on mortality. More accessible ways to do crucial in-centre investigations, avoiding travel costs and need of companions, which induce this non-compliance, should be provided.

P111 (EI0342)
VASCULAR ACCESS FOR HEMODIALYSIS PATIENTS IN SLOVENIA – DATA FROM THE SLOVENIAN RENAL REPLACEMENT THERAPY REGISTRY
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Objectives: To present data on vascular access of hemodialysis (HD) patients in Slovenia.
Methods: Data from the Slovenian Renal Replacement Therapy Registry, as of December 31, 2008, concerning vascular access are presented.
Results: There were 1343 prevalent chronic HD patients (population of Slovenia is approximately 2 million). 58% were men, median age 66 years, range 9-94, mean 63±15, 24.2% diabetics, median dry body weight 68kg. Median weekly duration of HD was 13.5 hours, mean blood flow 283±51mL/min, 123 (9.2%) patients were dialyzed by single-needle dialysis mode. Vascular access were: native arteriovenous fistula (AV) in 82.4% (n=1107), PTFE graft in 5.5% (n=74) and HD catheter in 12.1% (n=162). Position of fistula/graft was on forearm in 66%, elbow/arm in 33% and on thigh in 1% of patients. HD catheters (n=162) were: temporary (non-cuffed) in 96.3% (n=156) and permanent silastic in 3.7% (n=6) of patients; precurved jugular in 78%, subclavian in 18% and femoral in 4%; single lumen in 80% and double-lumen in 20%. 30% citrate locking was used in the majority of catheters. The most common type of catheter used was precurved non-cuffed 8F single-lumen jugular catheter (Medcomp, Harleysville, PA, USA), either as single or two catheters in the same vein. There were 224 new HD patients in 2008, who were alive and on HD on December 31, 2008, median age 67.5 years, 29.5% diabetics. Their vascular access at the end of 2008 were AV fistula in 68%, graft in 3% and HD catheter in 29%. All catheters were non-cuffed.
Conclusions: 82.4% of prevalent and 68% of incident HD patients in Slovenia had native AV fistula at the end of 2008. The vast majortiy of HD catheters used were non-cuffed single-lumen catheters with citrate locking, both for temporary as well as long-term use.

P112 (EI0341)
VASCULAR ACCESS ACTIVITY IN DIALYSIS CENTER UMC LJUBLJANA
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Objectives: To present vascular access activity in the Dialysis center of the University Medical center Ljubljana (UMCL).
Methods: Data on hemodialysis catheters insertions, arteriovenous fistula (AVF) and graft constructions and revisions and Doppler ultrasonography examinations was evaluated from in-center medical archive.
Results: Nephrologists in Dialysis center have constructed AV shunts from 1974-1982, AVF and grafts from 1974, inserted hemodialysis catheters from 1976, and performed Doppler-ultrasonography related to AVF (preoperative mapping, Doppler of dysfuntional or thrombosed AVF and grafts) from 1992. Nephrologists also perform reconstruction and surgical salvage of the suddenly thrombosed AVF and graft. Pediatric AVF were also created. Surgeries are performed in the operative theatre at the Dialysis Center, mostly under local anesthesia, as outpatient procedure. In a few patients (mainly pediatric) AVF was created under general anesthesia. A total of 6,172 AVF/graft surgeries (creations/salvage procedures) and 21,740 of hemodialysis catheter insertion were performed by nephrologists in Dialysis Center UMCL from 1974 until the end of April 2011. In 2009, 1,187 catheters were inserted, 267 AVF/grafts surgeries and more than 400 Doppler examinations were performed. The reason for the great number of catheters was in using 2 single lumen catheters instead of 1 double lumen catheter and providing the vascular access in the patients in intensive plasmapheresis (acute and chronic) and ICU (acute) therapy.
Conclusions: From 1974 to April 2011 nephrologists performed 6,172 vascular access surgeries, mainly AV fistulas and grafts (re)constructions and inserted 21,740 HD catheters, with approximate yearly vascular access activity of 250 surgeries, 800 hemodialysis catheters insertions and 400 Doppler examinations.

P113 (EI0215)
ARTERIOVENOUS FISTULA IN PEDIATRIC PATIENTS WITH END-STAGE RENAL DISEASE: THE ROLE OF PREOPERATIVE ULTRASONOGRAPHY MAPPING
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Objectives: To report our experience with ultrasonography preoperative mapping of arm and forearm vessels before arteriovenous (AV) fistula construction in pediatric patients.
Methods: Between 2002 and 2011 preoperative ultrasonography examinations were performed in 24 pediatric patients with end-stage renal disease (ESRD), 15 boys and 9 girls, aged 9-21 years. Arteries and veins of both arms and forearms were examined by ultrasonography/Doppler pre-operatively. Inner diameter of veins (under compression) and arteries and peak systolic velocity (PSV) were measured. Optimal position for AV anastomosis was suggested. If veins and/or arteries were not optimal, 8-week handgrip training was prescribed before the operation. AVF was constructed by trained nephrologists, under local or general anesthesia.
Results. Suitable veins on right forearm were recorded in 21/24 patients, with internal diameter of 2.9±0.7mm. On the left forearm suitable veins were found in

22/24 patients, measuring 3.2±0.6mm. Internal diameter of right radial artery was 1.6±0.3mm, of the left 1.7±0.3mm, PSV 22±6cm/s on the right and 24±5cm/s on the left. In all patients AVF was constructed, in all but three constructions it was successful. Two patients with unsuccessful forearm AVF construction had suboptimal forearm vessels, one of them was on steroid therapy. In the third patient, there was a spasm of the radial artery during operation, and after two primary failures in the arm, forearm AVF was successfuly constructed. On 1st of January 2011, 17/24 AVFs were still functional, double-needle dialysis was performed with average blood flow 256±53 mL/min. Two patients were dialyzed using catheter (in one patient AVF did not mature and in one patient forearm AVF clotted). 5/24 patients had a functional kidney transplant (deceased donor).
Conclusions: Ultrasonography can successfully assess arteries and veins of arms and forearms in pediatric ESRD patients, suggesting the optimal position for arteriovenous anastomosis, resulting in low primary failure rate.

Polymeric Membranes/Blood Interfaces

P115 (EI0362)
NEW MOULDING TECHNIQUE FOR TISSUE ENGINEERING OF FIBRIN-BASED AUTOLOGOUS AORTIC HEART VALVES
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Objectives: The presence of the sinuses of Valsalva in the aortic root is fundamental for the correct functioning of the aortic valve. Still the implementation of these features is not standard in tissue-engineered valves. In our laboratory we aim at the realization of autologous heart valves starting from materials (fibrinogen and cells) isolated from the patient and shaped into 3D geometries-like valved conduits with three leaflets by moulding techniques. However, when the diameter of the conduit is not constant as in the case of the aortic root including the sinuses of Valsalva, these moulding techniques cannot be straightforwardly applied and new concepts must be developed. We present a new fabrication method relying on a collapsible inner part of the mould that results in the realization of a heart valve scaffold recreating the complex geometry of the aortic valve.
Methods: The new mould was designed with the 3D CAD software Pro/Engineer (PTC, Needham, MA, USA) and manufactured by rapid prototyping. The collapsible part of the mould was made of silicone rubber. The fibrin gel valves were produced by polymerizing a fibrinogen solution in TBS (10mg/mL) with $CaCl_2$ and thrombin**.**
Results: After the polymerization of the fibrin gel had occurred, the inner part of the mould was collapsed and the construct was successfully released without any tearing despite the poor mechanical properties of the hydrogel. We obtained a conduit presenting a three-leaflet valve and the sinuses of Valsalva cast as a single piece without the need for suturing any of the parts together. Ongoing research is focused on the culturing and evaluation of the scaffold *in vitro*.
Conclusions: Implementation of the sinuses of Valsalva is a crucial step towards the development of functional tissue-engineered heart valves with optimal hemodynamic performance and reduced risk of thrombus formation.

P116 (EI0356)
ENDOTHELIZATION OF ARTIFICIAL BLOOD VESSELS INSIDE LARGE BODIES OF BACTERIAL CELLULOSE HYDROGELS
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Objectives: Macroscopic bodies of bacterial cellulose hydrogel with internal channels that mimic blood vessels were developed in our laboratories. The ability of human endothelial cells (HUVECs) to form an endothelial layer *in vitro* within these channels was examined.
Methods: The characterization of macroscopic bodies of bacterial cellulose hydrogels was performed by scanning electron microscopy (SEM). SEM was also used to evaluate the adhesion and proliferation of cells in the biomaterial; cell viability was evaluated by MTS and Live/Dead® assays. The endothelization of internal channels of the hydrogel was observed by confocal microscopy by revealing HUVEC actin filaments (Alexa Fluor 546 phalloidin) and cell nuclei (DAPI).
Results and Discussion: The formation of internal channels in the bacterial cellulose hydrogel was confirmed by SEM analysis. Channel walls are characterized by a high density of cellulosic fibers in the lumen side and a highly porous fibrous network in the surrounding hydrogel matrix. Seeded HUVECs adhered on the internal hydrogel channels and remained viable and proliferated for 26 days *in vitro*. By confocal microscopy we observed the formation of an endothelial layer on the lumen side.
Conclusions: The internal channels formed within the macroscopic bacterial cellulose hydrogel can be an excellent platform for artificial blood vessel studies. The endothelization of these channels has proven its ability to maintain cell viability *in vitro* and can potentially be the basis of several biomedical devices development.

P117 (EI0338)
LIPOSOME-ENCAPSULATED HEMOGLOBIN AMELIORATES SKELETAL MUSCULAR ISCHEMIA AND REPERFUSION INJURY IN THE RAT
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Objectives: Liposome-encapsulated hemoglobin (LEH), a cellular artificial oxygen carrier, has been reported to ameliorate vascular ischemia and reperfusion injury in various organs. In the current study, LEH with low O_2 affinity (l-LEH, $P_{50}O_2$=45 mmHg) was tested in acute disruption of limb perfusion as a simulation of thrombotic and/or atherosclerotic occlusion of the limbs, involving skeletal muscular ischemia and reperfusion.
Methods: Physiological parameters, such as blood pressure, heart rate, respiratory rate, tissue perfusion by Laser-Doppler flow meter as well as intramuscular PO_2, were serially monitored using dual PO_2 electrodes in the bilateral hind limbs in the SD rat. After baseline measurements, the left hind limb underwent ischemia by tightening a tourniquet for 70min, followed 10min later by intravenous administration of l-LEH (10mL/kg, n=4) or saline (n=4) to the tail vein. Reperfusion was effected by relaxing the tourniquet and the rat was followed for an additional 50min. Animals were sacrificed 7 days after ischemia/reperfusion for morphological analyses.
Results: While PO_2 decreased precipitously after the onset of ischemia in the ischemic limb regardless of treatment, the PO_2 value in the contralateral intact limb showed a different pattern; decreased in the LEH-treated rats and increased in the saline-treated control animals. As the result, PO_2 ratio in the left ischemic limb to the right non-ischemic limb (Lt/Rt ratio) tended to be higher during ischemia and became significantly higher in the LEH-treated rats than in the saline-treated control animals immediately after reperfusion to the end of observation. While plasma lactate, blood gases or electrolytes showed no difference after reperfusion, pathological studies 7 days later showed better muscular preservation in LEH-treated animals.
Conclusions: The results suggest that early LEH-treatment may be protective of the skeletal muscle after ischemia and reperfusion in the rat.

P118 (EI0334)
AN ATRAUMATIC MINI-PUMP FOR PULSATILE FLOW IN BIOREACTORS
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Objectives: Multiple types of bioreactors with perfusate are used for the culturing and examination of cell cultures and their interaction with the perfusion media. In case of use with full blood or corpuscular blood components as perfusion medium, the traumatization of the necessary pump is a crucial aspect. The usual roller pumps create unacceptable trauma and activation, due to the low priming volume and the multiple exposition of the blood particles to the pump shear.
Methods: Multiple types of bioreactors with perfusate are used for the culturing and examination of cell cultures and their interaction with the perfusion media. In case of use with full blood or corpuscular blood components as perfusion medium, the traumatization of the necessary pump is a crucial aspect. The usual roller pumps create unacceptable trauma and activation, due to the low priming volume and the multiple exposition of the blood particles to the pump shear.
Results: First tests showed a hemolysis increase for the MP of only 1.4 compared to 25.3 mg/dL/hour for the RP. ICAM-1, HLA-DR and CD11b were moderately activated by MP, but massively by RP. In contrast to RP, MP did not cause

the expression of procoagulatory tissue factor on monocyte surface.
Conclusions: A minipump with very cheap disposable components could be developed, which caused very low blood trauma in first tests.

P119 (EI0310)
BIOFUNCTIONALIZATION OF BLOOD-CONTACTING MATERIALS FOR *IN VIVO* ENDOTHELIALIZATION
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Objectives: Biofunctionalization of blood-contacting materials with endothelial progenitor cell (EPC) specific capture molecules represents an auspicious strategy for *in vivo* tissue engineering of an endothelium on artificial implants.
Methods: For this purpose, aptamers, antibodies, peptides, or magnetic molecules can be used as capture molecules for EPCs.
Results: Synthetic graft surfaces coated with capture molecules for EPCs mimic a pro-homing substrate to fish autologous EPCs directly out of the bloodstream. After implantation, EPCs with high proliferation potential can cover the graft with a non-thrombogenic endothelium, which maintains optimal hemostasis and minimizes the risk of restenosis.
Conclusions: The realization of this *in vivo* tissue engineering concept and the generation of an endothelium on artificial surfaces could exceedingly enhance the performance of not only small caliber vascular grafts and stents, but also, in general all blood-contacting medical devices, such as heart valves, artificial lungs, hearts, kidneys, and ventricular assist devices.

P120 (EI0309)
OPTIMIZATION OF HEMOCOMPATIBLE STENT COATING USING POSS-PCU NANOCOMPOSITE POLYMER
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Objectives: In-stent restenosis (ISR) is an unresolved issue in the field of interventional cardiology leading to vessel obstruction, reoccurrence of clinical symptoms and high mortality rates in a significant number of patients. Polymer coating of bare metal stents (BMS) has been applied with the intention of increasing stent patency rates and prolonging their maximum effective lifetime. In this study, we address key factors that directly influence the polymer coated stent performance, namely the selection of polymer, the applied coating technique and the adhesion of polymers to the stent surface.
Methods: Cobalt Chromium BMS were coated with hemocompatible POSS-PCU using the ultrasonic atomization spraying technique. Surface coating procedures were optimized. Chemical modification and pre-polymer coating was applied for covalent modification of the BMS to improve polymer adhesion. The stress-strain behavior, surface morphology, and wettability of 100 µm thick sprayed and casted POSS-PCU films were compared. Peeling tests and cyclic balloon expansion studies were evaluated. Pulsatile fatigue studies were performed over 400 million cycles, stimulating 10 year *in vivo* study, following ISO international standards.
Results: Uniform surface coatings were achieved at 10mm in thickness. Contact angle (θ) measurements show that POSS-PCU was more hydrophobic (θ = 96.7±2.47°) than BMS (θ = 69±5.56°). Evaluation of surface chemistry using ATR-FTIR showed no change in chemistry of the polymer after spraying. Peeling tests show a significant improvement in BMS-polymer adhesion after modification ($p<0.0001$). Stress-strain behavior revealed that maximum tensile stress was significantly lower for sprayed POSS-PCU ($p<0.0001$) when compared with controls. SEM morphological assessments of the stent surface demonstrated that the spray coated stents maintained their physical integrity after fatigue studies.
Conclusions: The optimization of BMS coatings shows great potential in the development of new generation of high patency rate coronary artery stents and for coating of medical devices.

P121 (EI0290)
MORPHOLOGY AND FUNCTION OF SMOOTH MUSCLE CELLS ON POLY(N-BUTYL ACRYLATE) NETWORKS WITH DIFFERENT ELASTIC MODUL
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Objectives: Small calibre vascular prostheses still lack medium and long-term patency. Inelasticity of the bulk material is one of the characteristics which are implicated in the mechanisms of failure. Here we report about poly(*n*-butyl acrylate) networks (cP*n*BA) with adjustable elastic moduli, which can be tailored to match the E-modul range of human arteries (between 200 and 1100 kPa). In view of vascular protheses as potential application of cP*n*BAs, viability and functionality of primary human vascular smooth muscle cells (VSMC) on cPnBAs were explored.
Methods: cP*n*BAs were synthesized by free radical polymerization. The elasticity was adjusted by varying the molar ratios of *n*BA monomer and polypropylene glycol dimethacrylate (PPGDMA) crosslinker. *In vitro* tests for cell viability were performed with VSMC (culture period of 96 hours). Deposition of VSMC extracellular matrix (ECM) proteins was quantified immunochemically. Secretion of cytokines was analyzed in a multiplex cytokine profile.
Results: Two soft hydrophobic cP*n*BA networks with E-moduli of 200 and 1100 kPa (surface roughness in the wet state of 17 and 37 nm; $\theta_{advancing}$ of 123±4° and 111±4°) were investigated. All *in vitro* tests suggested a decreased viability of the VSMC compared to the control, unaffected by the elastic modulus of the cP*n*BA. Furthermore, ECM deposition was decreased. The cytokine profile showed a shift towards the synthetic phenotype and, moreover, indicated a pro-inflammatory response of the VSMC to the cP*n*BAs.
Conclusions: The results of this study demonstrate the challenge in the development of multifunctional materials. While the elastic modulus of cPnBA-networks could be successfully adjusted to that of human arteries, the tested polymers did not show an optimal performance as substrate material for VSMCs. Future studies aim at improving the biofunctionality by surface modification of these polymer networks.

P122 (EI0269)
HEMOCOMPATIBILITY OF POSS-PCU NANOCOMPOSITE POLYMERS
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Objectives: There is increasing demand for synthetic polymeric materials that resist thrombosis for cardiovascular biomaterial applications. Recent advances in nanotechnology and polymer chemistry have led to enhanced physiochemical and mechanical properties of nanocomposite materials. In this study, we investigate the composition of nanomaterials by covalent attachment of polyhedral oligomeric silsesquioxane (POSS) nanocages into poly(carbonate) urethane (PCU) to evaluate their effects on the coagulation cascade, platelet response and whole blood to evaluate hemocompatibility.
Methods: POSS was reacted with PCU to form nanocomposite materials ranging from 2 to 8% w/w. Polymer films were manufactured into circular discs (16mm in diameter). PTFE films were kindly supplied by Porex Technologies Ltd and used as a simple model control. Citrated whole blood was collected from healthy volunteers and centrifuged to isolate platelet rich plasma (PRP). Whole blood studies were conducted to evaluate thrombus formation through thromboelastography (TEG) to monitor blood clot development, kinetics and lysis.
Results: Morphological studies of adhered platelets on PTFE were composed mainly of spreading pseudopodia, dendritic structures and fully flattened activated phenotypes. On 2% POSS-PCU films only rounded platelets were apparent with no distinct pseudopodia. Whole blood studies revealed that clot formation was significantly reduced and less stable on 2% POSS-PCU films. TEG analysis also revealed a delay in clot formation, decrease in clot strength and increase in clot lysis when compared with controls.
Conclusions: Covalent modification of POSS within PCU led to a distinctive anti-thrombogenic response. 2% POSS-PCU prevented platelet adhesion, aggregation and activation with a reduced rate of clot formation, stability, and increased lysis. This may be related to surface topography, chemistry, energy or charge on 2% POSS-PCU films. Such nanocomposite materials can be used for cardiovascular applications where thrombosis needs to be minimized and is currently undergoing pre-clinical evaluation following GLP protocols.

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P123 (EI0199)

ANALYSIS OF CERAMIC AND POLYMERIC MEMBRANES USING FLUORESCENT-LABELED ENDOTOXIN

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Objectives: Endotoxins are components of the bacterial cell wall and are found in almost all fluids even in those which are poor of nutrients. This is a huge problem for medical and pharmaceutical applications, because already small amounts of endotoxin cause strong immune reactions. Endotoxin removal is possible by e.g. filtration. Therefore normally polymer membranes are used. The disadvantage of these membranes is their short life time. An alternative are ceramic membranes because they are inert and long-lasting. This work investigates polymeric and ceramic membranes concerning their ability of endotoxin removal from aqueous solutions e.g. dialysis water. Beside the analysis of endotoxin removal with conventional LAL-test fluorescence-labeled endotoxins and microscopic analysis of the membranes have been done.
Methods: Several polymeric adsorber membranes and ceramic membranes were investigated concerning their endotoxin removal ability. Therefore membranes were loaded with aqueous endotoxin solutions (0-1000 EU/mL) and tested in cross-flow and dead-end modus. The permeat samples were analyzed by the Limulus Amoebocyte lysate (LAL) test. The endotoxin removal of a membrane was classified as sufficient at permeat endotoxin levels under 0.25 EU/mL. For microscopic analysis the membranes were loaded with fluorescence-labeled endotoxin, embedded and analyzed via fluorescence microscopy.
Results: Although adsorber membranes showed good endotoxin binding capabilities the endotoxin removal was insufficient. Ceramic membranes showed significant better endotoxin separation. Microscopic analysis showed that in polymeric membranes endotoxin could penetrate until a depth of 25µm, whereas in ceramic membranes the penetration depth was only 4µm.
Conclusions: Microscopic analysis of endotoxin filtration gives a deeper understanding in the separation behavior of the investigated membranes. Further fouling and capacity of the membranes can be observed directly. In the future a quantitative correlation of the endotoxin amount on the membranes should be established by measuring the fluorescence intensity of the labeled endotoxin.

P124 (EI0197)

ELASTOMERIC PHOTOPOLYMERS BY THIOL-ENE POLYMERIZATION FOR VASCULAR TISSUE ENGINEERING

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Objectives: Diseases of the cardiovascular system account for a significant number of morbidities and mortalities in developed counties. Hence, the need of suitable materials for artificial replacements for damaged blood vessels arose over the last decades, especially in the field of narrow blood vessel substitutes. This research focuses on the fabrication of blood vessel substitutes based on elastomeric photopolymers.
Methods: Additive manufacturing technologies (AMTs) such as digital light processing are very capable techniques for the fabrication of constructs with complex geometries and high resolution mimicking the cellular structures of biological materials. For the application as blood vessel substitutes, polymers possessing urethane groups are interesting candidates since they exhibit elastic properties and also show good biocompatibility. Various urethane oligomer acrylates were tested in combination with different monofunctional acrylates as reactive diluents. In order to match the material properties of native blood vessels, a combination of low crosslink density and high urethane group concentration was desirable. To accomplish this, dithiols were added to formulations allowing the resulting thiol-ene reaction to compete with acrylate homopolymerization and thus lower crosslink density.
Results: The high content of urethane groups caused a high density of reversible crosslinks due to H-bonds. With this polymer architecture the material had elastomeric properties comparable to native vascular tissue and exhibited similar tensile strength and suture tear resistance tests. Due to the hydrolytically cleavable ester bonds along the back bone and the branches, these polymers possess an inherent degradability similar to that of poly(lactic acid). The polymers also exhibited good endothelial cell attachment, which is crucial for the long-term performance of the vascular grafts.
Conclusions: Structuring photopolymers by means of AMT enables the fabrication of artificial grafts with complex geometries. Implementation of the thiol-ene concept leads to materials with both highly elastomeric mechanical behavior and good biocompatibility.

P125 (EI0368)

2D BI-LAYER SCAFFOLDS OF POLYCAPROLACTONE AND CHITOSAN β-GLYCEROL-BASED FILM FOR BLOOD VESSEL CONSTRUCTS

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Objectives: The objective of this study was to develop a scaffold model aiming at fabricating small diameter blood vessel grafts with distinct surface properties. This study was designed to evaluate the influence of the scaffold properties on endothelial and smooth muscle cells.
Methods: The scaffolds consisted of either a polycaprolactone (PCL) nanofibre mesh (NF) layer fabricated by means of electrospinning or a PCL membrane fabricated by solvent casting (SC); and a second layer prepared from a mixture of β-glycerol phosphate salt (GP) and chitosan (Ch). Scaffold characterization was performed in terms of surface topography (SEM) and mechanical properties (tensile, Young's tensile and yield stress; and strain at break). For the biological evaluation endothelial and smooth muscle cells isolated from the vein of human umbilical cord (HUVECs and HUVSMCs) were used. Single cell cultures were established for both cell types and both scaffolds up to 7 days. Cell behavior was evaluated after DNA quantification, alkaline phosphatase activity, methylene blue staining and SEM.
Results: The tensile strength values for both SC PCL and NF PCL scaffolds exceeded the one of natural artery (15MPa vs. 3MPa vs. 1MPa). As expected no alkaline phosphatase activity was detected in the cultures. Moreover, HUVECs attachment and proliferation rate was significantly higher on the SC PCL layer while for HUVSMCs the opposite was observed and the NF PCL layer was the preferable substrate for adherence and growth.
Conclusions: Scaffolds with mechanical properties capable of withstand the physiological vascular conditions were obtained. The GP layer did not cause any sign of calcification, which constitutes a good indicator for its incorporation within the blood vessel scaffold. The selective response of each cell type to a specific surface topography allows the definition of the design of a blood vessel graft combining HUVECs and HUVSMCs in the opposite layers.

P126 (EI0270)

ORIENTATION OF ELECTROSPUN FIBERS BY MINIMIZING JET INSTABILITIES

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Objectives: Basic electrospinning setups suffer from random fiber deposition conditions thus limiting their applications. The aim of this work was to minimize the bending instability of the jet in order to produce scaffolds with predesigned geometries.
Methods: Polyurethane was electrospun on a horizontal oscillating and rotating conductive aluminum mandrel. The required electrostatic field essential to overcome the surface tension of the polymer solution was complemented by an auxiliary gradient field. This was generated by two additional electrodes that were symmetrically positioned around the spinning nozzle and operated with adjustable high voltage. The effect of the auxiliary electric field was characterized by comparing fiber deposition at different surface velocities, spinning times, with and without auxiliary electrodes.
Results: Without the auxiliary electrodes only poor fiber alignment was possible. By introducing the auxiliary gradient field it was possible to minimize jet instabilities and improve fiber alignment. Fiber deposition took place in a focusable plane between the auxiliary electrodes. After 5 minutes' spinning on a rotating mandrel the fiber deposition could be focused to a 3mm width area. Oriented fiber deposition was achieved with the auxiliary electrodes at target velocities starting from 1m/s for the first deposition layer. For longer lasting spinning durations surface velocities up to 6m/s were necessary to achieve aligned and straight fibers.

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Conclusions: The bending instability, one shortcoming of electrospinning controllability, could be considerably reduced.

Clinical VADs and Balloon Pumps

P127 (EI0378)

PREDICTIVE ROLE OF SERUM CYSTATIN C (SCYS C) ON SURVIVAL IN CARDIAC SURGICAL PATIENTS

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Objectives: Pre-operative renal dysfunction is a known risk factor for morbidity and mortality in cardio-surgical patients. Serum Creatinine (sCr) is the only renal marker in the pre-operative scores, but it is known its diagnostic limitations on CKD. sCys C is a well-recognized marker of early renal dysfunction, and previous studies suggest a sCys C predictive role for cardiovascular events in the general population. However, few reports evaluate the prognostic role of sCys C in cardiac surgical patients. Aim of this study is to assess the long-term (two years) prognostic value of sCys C on mortality in adult cardiac-surgical patients.
Methods: 421 consecutive patients (250 male and 171 female, mean age 67.72±10.76 years), inpatients in the cardio-surgery department from November 2005 to March 2007, were enrolled. We conducted a prospective observational study evaluating all causes of mortality until December 2009. At admission all patients were tested for renal function by sCr and sCys (normal value 0.5-0.92mg/L). 217 out of 421 were submitted to coronary artery bypass graft (CABG), 150 valvular prostheses, 54 for other kinds of cardiac surgery. Patients were subdivided in quartiles according to their sCys C values: Q1 sCys C<0.81mg/L (29 patients), Q2 sCys C 0.81-0.92mg/L (81 patients), Q3 sCys C 0.93-1.10mg/L (29 patients) and Q4 sCys C>1.10mg/L (282 patients). Kaplan–Meier cumulative survival curves were plotted for sCys C quartiles.
Results: 124 patients (29.4%) reached the study end-point. Patients in Q3 and Q4 showed a higher cumulative probability of mortality compared to patients in the lowest quartiles (p=0.0007).
Conclusions: Increased levels of serum Cystatin C may be considered a predictor of cardiovascular mortality at two-year follow-up in cardiac-surgical patients.

P128 (EI0286)

MINIMALLY INVASIVE IMPLANTATION OF A PARA-AORTIC BLOOD PUMP

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Objectives: Mechanical support is the only way that is effective for breaking the vicious cycle of the heart failure mechanism. However, the decision of mechanical intervention via ventricular assist device (VAD) implantation has been deferred to end-stage heart failure because of the invasiveness and high mortality/morbidity rate of the surgery. Besides device efficacy, earlier intervention holds the key of reversing the trend of pathological myocardial remodeling. A counterpulsatile para-aortic blood pump (PABP) was invented to achieve this goal of early intervention.
Methods: By taking advantage of the semi-rigid property of this PABP, a minimally invasive surgery (MIS) method was devised. This MIS procedure can be conducted using left thoracotomy without cardiopulmonary bypass support. A small incision size of 5-7cm at the 5th and 6th intercostal space is required for the insertion of the PABP device. The implant site on the descending aorta was secured by a two-sided cross-clamping and the ligation of spinal arteries was done to free the aorta. A tool kit comprises a hole-maker, an insertion holder, a quick-release pouch, and an endoscope for visual monitoring was designed and constructed. With the aid of these tools and a specially designed surgical protocol, the present PABP can be implanted quickly requiring only 3-5 minutes ischemic cross-clamping time. A bandage-type perivascular fastener was also invented and used to rule out the conventional need of anastomotic suturing.
Results: It was shown on the animal model that this MIS PABP implantation can be accomplished safely and quickly and no bleeding complication was observed.

P129 (EI0283)

PRECLINICAL *IN VIVO* AND *IN VITRO* INVESTIGATIONS OF A THROMBORESISTANT PARA-AORTIC COUNTERPULSATILE DEVICE FOR LONG-TERM VENTRICULAR SUPPORT

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Objectives: A para-aortic blood pump (PABP) intended for long-term counterpulsatile circulation support has been developed. This report summarizes the preclinical *in vivo* and *in vitro* tests that have been conducted for examining the hemodynamic performance and efficacy of this newly designed PABP.
Methods: Acute porcine heart failure model induced by coronary ligation was adopted, and the hemodynamic support efficacy of PABP (n=8) was evaluated using intra-aortic balloon pump (IABP, n=8) as a benchmark. The chronic tests, designed to test the hemocompatibility and the integrity of the implanted device to major end-organs of the recipients, were conducted using healthy calves (n=5). No anti-coagulants were administered during the post-operative 3-month period. To further look into the detail pulsatile blood pump flow field, a flow visualization experiment was launched *in vitro*. Particle tracers were released to map and characterize the entire unsteady flow field.
Results: When compared to IABP, PABP possesses better hemodynamic performance in every measured hemodynamic and metabolic index. For chronic tests, at autopsy, no clots were found on the pump surface and all recorded monitoring parameters including blood and organ functions are found normal, indicating the thromboresistance and biocompatibility of the PABP implant. The flow visualization using Lagrangian particle tracing reconfirms that no stasis zones exist and an excellent vortex wash-out effect was achieved in the blood pump chamber.
Conclusions: Based on these *in vivo* and *in vitro* test results, it is concluded that the present PABP makes a promising counterpulsatile modality that is thromboresistant and therapeutic, and hence can be considered in the future as a viable long-term implantable ventricular assist device.

P130 (EI0159)

PRESSURE MEASUREMENTS ALONG THE INTRA AORTIC BALLOON (IAB): IMPLICATIONS OF OPERATING AT AN ANGLE TO THE HORIZONTAL

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Objectives: The aim is to investigate the operation of intra aortic balloon (IAB) at angles to the horizontal, resembling patients being nursed at semi-recumbent positions.
Methods: Two Datascope balloons, 34cc and 40cc, have been tested in a mock loop at 0,° 20° and 30°. Pressure at 7 positions along the balloon, and flow-rate on either side of the balloon during inflation and deflation were sampled simultaneously at 2kHz. The ratio (RQ) of water volume displaced towards the tip to the total volume displaced, pressure pulse generated by balloon inflation (PP) and the time of maximum pressure (TMP) at each location were determined.
Results: At 0° TMP was reached at the tip and base of the 34cc balloon almost simultaneously. At 20° and 30°, TMP was reached at the tip earlier than the base by 4ms and 3ms respectively. RQ at 0°, 20° and 30° are 51.5%, 48.8% and 49.6% respectively. TMP for the 40cc balloon was reached at the tip earlier than the base by 4ms, 4.5ms and 6ms at 0°, 20° and 30°, respectively. This was associated with a decrease in RQ from 49.7% to 45.9% and 44.4% at 0°, 20° and 30°. Maximum PP along the balloon decreased by 12.57% and 18.6% at 20° and 30° for the 34cc balloon and by 17.42% and 27.93% for the 40cc. This was associated with a decrease in total volume displaced (TVD) of 2% and 21% at 20° and 30° (34cc balloon) and 21% and 36% (40cc balloon).
Conclusions: TMP occurring earlier at the tip than other sections of the balloon provides a possible explanation on the reduction of RQ when balloons operated at angles to the horizontal. Efficacy of balloons maybe compromised when operated at angles to the horizontal with a reduction in both PP and TVD.

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P131 (EI0238)

STABILIZATION OF CARDIAC FUNCTION AFTER EPICARDIAL BIOGRAFT IMPLANTATION

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Objectives: Progress in cardiac tissue engineering is conditioned by the creation of a suitable environment for cells to build up an organised tissue. Hence, substrates need to be architecturally and chemically tuned to match the destination tissue. In this regard, micro-fibrous matrices, enriched with oxygen functional groups were designed and seeded with bone marrow derived mesenchymal stem cells (MSCs). In a rat *in vivo* model, the hypothesis that epicardial implantation of a cell-polymer graft has beneficial effect on cardiac function was tested.
Methods: Microfibrous PCL non-wovens were produced by electrospinning and surface-coated by an RF plasma process (CO_2/C_2H_4 gas). MSC were characterized by FACS and 2 Mio cells were cultured for 7-10 days on the fibrous patches (10*15mm). Cell mortality was assessed by LDH release, viability and morphology by MTT staining and SEM imaging, respectively. Two weeks post LAD ligation, Lewis rats with reduced ejection fraction (EF of 48±8%) were randomised into 4 groups: MSC-seeded patches glued onto the infarcted area with Tisseel fibrin glue (n=7), cell-free patches (n=8), glue only (n=4) and sham operation (n=5). Echocardiography and pressure-volume loops were recorded after 28 days. Histological analyses are under investigation.
Results: CD90+, CD45- and CD31- MSC spread on the matrix, producing a homogenous monolayer. The biografts allowed for safe implantation without signs of rejection, encapsulation or inflammation. Patches were permanently glued onto the myocardium, without adhesion to other organs. Relative to pre-treatment, MSC-seeded patches induced an EF stabilisation after 4 weeks (48±10% and 48±7% respectively). Cell-free patches resulted in a significantly decreased EF (45±9% and 39±4% respectively, p=0.05).
Conclusions: Preliminary data on EF analysis demonstrate that epicardial implantaion of plasma coated, MSC-seeded PCL biografts allow for safe implantation and attenuate cardiac remodelling. Further analysis will confirm eventual effect on myocardial regeneration and characterise macrophage invasion and vessel formation.

P132 (EI0219)

LARGE ANIMAL MODELS OF HEART FAILURE FOR DEVELOPMENT OF NEW LVAD THERAPY: COMBINATION METHOD OF MICRO-EMBOLIZATION AND RAPID PACING CAN CONTROL THE DEGREE OF HEART DYSFUNCTION AND STABILIZE THE RESULT OF MODEL MAKING

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Objectives: To develop the new LVAD therapy for heart failure it is very much important to establish the method of making large animal model of heart failure. But unfortunately it is so difficult to make the model steadily with well-controlled degree of heart failure. Now we have challenged to make large animal model with ischemic heart failure, more successfully by combination method of micro-embolization and rapid pacing.
Methods: We studied an adult female goat (60kg). From a multipurpose catheter introduced into the left carotid artery toward left anterior descending coronary artery (LAD), we injected the microsphere (size50μm) into LAD 0.225million (3,750/kg). 2days later, we started rapid pacing (heart rate 200) and continued for 2 months. Aortic blood pressure (AoP), central venous pressure (CVP), arterial pressure-based cardiac output (=APCO, Edwards Life Science LLP, Irvine CA, USA) were monitored. We performed echocardiography as needed.
Results: AoP was decreased from 120/80mmHg to 90/70mmHg, CVP was elevated from 6mmHg to 15mmHg, and APCO was decreased from 4.8L/min to 2.8L/min. She lost appetite, and was racked (roughly equivalent to NYHAIII). But 2 days after, the general condition was returned to NYHAIII, and APCO was around 3. So we started rapid pacing, and cardiac function remained low. By echocardiography, wall motion was hypokinetic in anterior wall and ventricular septum, ejection fraction (EF) was decreased from 80% to around 50%, and LVDd/Ds were dilated from 32/14mm to 48/32mm. Pathologically, there were many small areas of necrotic myocardium in subendocardium.
Conclusions: By changing the rapid-pacing start timing and rate, according to the heart function after the micro-embolization, we can control the degree of heart dysfunction, which we cannot achieve only with micro-embolization. And this may make the good survival rate of model making. This unique combination method of heart failure model-making may contribute for development of the new LVAD therapy.

P133 (EI0179)

EFFECT OF REGIONAL LEFT VENTRICULAR DYSFUNCTIONS ON CONTINUOUS FLOW LVAD ASSISTANCE

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Objectives: Patients with severe heart failure are candidates for LVAD implantation. Left ventricular (LV) dysfunction is related to abnormal ventricular region (AVR) extension that could be affected by systolic abnormalities, diastolic abnormalities and electro-mechanical dissynchrony. The aim of this work is to study the effect of an AVR on hemodynamics during LVAD assistance.
Methods: A lumped parameter model of the cardiovascular system was updated. Circulatory sections are described by Windkessel models. Atria, ventricular free walls and interventricular septum are described by variable elastance models driven by ECG times. LV free wall is represented by two variable elastance models to simulate an AVR. A model of parallel continuous flow LVAD was also implemented. Starting from a simulated pathological condition, the effect of LVAD on hemodynamics was studied changing: 1) AVR systolic elastance, 2) AVR diastolic elastance 3) the contraction delay between the two parts of the LV free wall and 4) AVR extension.
Results: All experiments compare the relative changes between pathological and assisted conditions giving their mean values. The variable that is more influenced by AVR changing is the external work. The presence of an AVR dissynchrony (0÷150ms) influences mean aortic pressure (+10%). The changing of AVR systolic function (0.1÷1 mmHg/mL) affects both LV end systolic volume (+6%) and cardiac output (+12%), while the changing of AVR diastolic function (0.01÷0.2 mmHg/mL) affects left atrial pressure (-19%) and LV end diastolic volume (-14%). The presence of an AVR diastolic dysfunction could expedite the occurrence of right ventricular heart failure. This fact is more evident if a diastolic septum dysfunction occurs together with a moderate AVR diastolic dysfunction.
Conclusions: The model could be useful to estimate the role of different parameters on LVAD performance and could be used to support the clinical decision adapting the LVAD assistance to specific patient conditions.

P134 (EI0027)

CIRCARDIAN VARIATION OF MOTOR CURRENT COULD BE OBSERVED IN FIXED ROTATION SPEED CENTRIFUGAL-CONTINUOUS FLOW LVAD SUPPORT

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Objectives: The algorism for physiological control has been controversial in the patients supported with continuous flow LVAD, in which supplied blood flow is regulated by the pressure gradient between aortic and left ventricular pressure when operated at constant rotation speed. Little has known relating physiological control algorism, such as achieving physiological circadian rhythm. Motor current was evaluated in terms of the existence of circadian variation in dilated cardiomyopathy patients supported with centrifugal-continuous flow LVAD.
Methods: The motor speed (rpm) and electric current (micro ampere) data were collected every 10 minutes after device implantation and were divided in every 30-day data and calculated by Least Spectrum method.
Results: Patients were 37.3±14.8 years old, weighed 63.8±15.1 kg at the time of operation. As of March 1, 2011, mean support duration was 1299.8 days (968 - 2124 days). Of the 6 patients, 3 received heart transplantation (at 1164, 1115 and 968 days of support) and 3 were still supported by LVAS with the mean support duration of 1517±535 days. The circadian variation of motor current was observed in all patients during almost entire course of LVAD support. The calculated periods of the circadian variation was 24.00±1.00 hours. The amplitude of the circadian variation was 11.48±4.50μA. The circadian variation of the motor current was tended to be relatively low during night time whereas that was tended to be relatively high during day time. There were significant night- and day-time variations (p<0.01)

Conclusions: Circadian rhythm of motor current could be observed in fixed rotation speed centrifugal-continuous flow LVAD support. The cause and effect of this variation are still unclear although it is speculated to be correlated to physiological changes of some hemodynamic-related parameters of patients.

P135 (EI0012)
CAN WE GENERATE SYSTEMIC ARTERIAL HYPERTENSION BY PULSATILE LVAD IN OUR PATIENTS?
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Objectives: On the basis of our personal experience of studying co-temporary scientific articles and the modelling of flow and pressure patterns in systemic vascular beds, we are encouraged to claim, that extremely small patients can experience systemic arterial hypertension generated by pulsatile LVAD.
Methods: Our aim is to present our research about "iatrogenic systemic arterial hypertension produced by a left ventricular assist device". We want to show articles in medical journals supporting these facts and demonstrate our software application for the modelling of flow and pressure patterns for the confirmation of our thesis. We are able to prove the first author's hypothesis that different sized vascular beds are adjusted for the appropriate stroke volume of the native heart and so blood circulation in different sized vascular beds must be supported by the pump with an appropriate stroke volume. Thoratec VAD uses only one pump chamber size for all patients with different vascular bed sizes (65mL). After implantation, all patients have the same stroke volume - 65mL, and the same average flow during the ejection period, i.e. 65mL for 300ms (13 L/min!). The average blood flow during the ejection period is higher in non-physiological terms for extremely small patients and may cause iatrogenic systemic arterial hypertension.
Conclusions: A safe and reliable ventricular assist device is a dream for many of us. Let's consider our work as a contribution for the better understanding of relationships between the human body and the mechanical device (VAD). We anticipate that the new generation of pulsatile VADs will come with adjustable stroke volumes and others parameters. The stroke volume expelled into the aorta must always be adjusted to the patient's size and actual requirements of the patient's body.

P136 (EI0194)
PHYSIOLOGICAL CONTROL OF AN LVAD TO CONTROL AORTIC VALVE MOTION IN THE HUMAN CARDIOVASCULAR SYSTEM
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Objectives: Continuous Flow Left Ventricular Assist Devices (CF-LVADs) generally operate at a constant speed in the patient. In this mode, they change the systemic blood flow waveform significantly. If the left ventricular pressure is continuously less than the aortic pressure the aortic valve (AV) remains closed over the cardiac cycle. Blood flows through the CF-LVAD from the left ventricle (LV) into the aorta and pulsatility of the blood flow decreases. In this study, blood flow through the AV is controlled by applying a feedback control to the CF-LVAD to generate intermittent flow through the AV.
Methods: PI control was applied to the flow rate through the LVAD in simulations. Minimum pump flow (Q_p) was set to 20 mL/s. Three flow ratios were simulated. Q_p being equal to 1/3 of AV flow (Q_{ao}), Q_p equal to Q_{ao} and Q_p equal to $3Q_{ao}$. The ventricle was beating at 80 bpm with 45% / 55% systole / diastole ratio.
Results: In the simulations the aortic valve duty cycle ($t_{open}/t_{cardiac\ cycle}$) did not change. However Q_{ao} changed significantly. The Q_p was controlled as desired by making the CF-LVAD operating speed variable over a cardiac cycle. The cardiac output value was 3.30 for all simulation protocols.
Conclusions: Simulation results show that the AV duty cycle does not change for the same cardiac output values. However, change in the amount of the flow rate through the aortic valve indicates that AV valve motion changes. In other words, without compromising total systemic perfusion the AV motion can be controlled by applying variable pump speed control. However, Q_{ao} has to be estimated because it cannot be measured directly in a patient. Experimental validation will be carried out as a next step.

P137 (EI0065)
OUTCOMES FOR CONTINUOUS-FLOW LEFT VENTRICULAR ASSIST DEVICE PATIENTS STRATIFIED BY SEVERITY OF CLINICAL STATUS
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Objectives: The use of continuous-flow left ventricular assist devices (LVAD) is an accepted therapy for patients with advanced heart failure. New generation of these devices may have an impact on improved survival rates and quality of life (QoL). Here we report about our single-center experience with the new generation of LVADs.
Methods: Between 2006 and 2010, 41 transplantable and 8 non-transplantable patients were selected for LVAD-therapy at our institution due to refractory severe heart failure. All patients were INTERMACS Level 1 to 3 (Level 1: n=23; Level 2: n=18 and Level 3: n=8). The cohort included 44 men and 5 women with a mean age of 53±12 (range 20-75 years). The patients were supported either by Heart Mate II (n=39) or HVAD (n=10) LVAD. In-hospital (30-day) and long-term survival, freedom form re-operation and neurological complication, and rate of drive-line infection were examined. Additionally, the QoL was assessed.
Results: Mean support time was 138±53 days (range 1-867 days). In-hospital (30-day) mortality was 27% (n=11) due to severe cardiogenic shock with multiple organ failure and sepsis. The follow-up survival for all was 32 of 49 patients (65%). Bleeding requiring reoperation occurred in 13 patients (n=27%). Neurological problems were identified in 12 patients. There were 6 drive-line infections. 63% of all patients were discharged at home. Overall, 3 patients underwent transplantation, 23 patients awaiting a donor organ, 1 patient was successfully weaned and finally, 5 patients are on destination therapy with good QoL.
Conclusions: Treatment of severe heart failure with new continuous-flow LVAD can significantly improve the acute and long-term survival with low device associated complication and improved QoL.

Tissue Engineering

P138 (EI0425)
NOVEL 3D MULTILAYERED CO-CULTURE SYSTEM FOR INVESTIGATING STEM CELL DIFFERENTIATION
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Objectives: To develop an ideal co-culture system for mesenchymal stem cells and articular chondrocytes. A number of recent studies show synergistic effects for chondrogenesis when the two cell types are cultured together, but there is still some ambiguity as to the specifics of these interactions. The critical paracrine signals and/or cell-cell adhesion modules involved must be identified. Via tailoring of the chemical and physical environment exposed to the cells in co-culture, ideal conditions will be identified for promoting the synthesis of cartilagenous tissue.
Methods: We intend to explore a novel technique for depositing layer-by-layer nanofilms of ECM molecules on individual cell surfaces to permit cell contacts analgous to native ECM interactions. QCM-D using adhered extensive work on polyelectrolyte multilayers and hydrogel encapsulation of cells are routinely carried out in our lab and will be incorporated into a "3D" co-culture system for the cells. Differentiation will be assessed with qRT-PCR, histology, and novel molecular reporters associated with up-regulation of chondro-specific genes. Cell types will be distinguished *in situ* via cell tracker molecules and/or other fluorescent labels.
Results: An effective, 3D co-culture system will be established for hMSCs and chondrocytes that will permit elucidation of the communication system between the two cell types. We expect to show that cartilage-specific genes are upregulated in constructs versus control groups.
Conclusions: A recent trend in literature concerning co-cultures involving adult stem cells is that, instead of promoting lineage specific differentiation, the stem cells rather provide support to improve the relevant function of the adult cells. This work will shed light on which soluble factors and/or cell-cell adhesions are involved in these processes and inform further tissue engineering strategies.

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P139 (EI0418)

INVESTIGATION OF SELF CROSS-LINKING POLYELECTROLYTES TO CONFORMALLY COAT INDIVIDUAL LIVING YEAST CELLS

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Objectives: One of the most promising applications of encapsulated living cells is their use as protected tissue for implantation in the human body. A suitable system for the immuno-protection of living cells is conformal coating. Layer-by-Layer assembly, a commonly used method for conformal coating, uses sequential deposition of alternating layers of positively and negatively charged polymers to coat materials with functionalized nanofilms. This permits the preparation of small capsules with minimal encapsulating material that helps to maximize metabolic exchange while minimizing overall capsule size. This work describes the use of auto cross-linking polyelectrolytes to coat individual living yeast cells. The effects of polymer properties such as molecular weight, concentration and compositions on cell coating and viability will be discussed and compared with non-cross-linked analogs. We also report preliminary results on encapsulated yeast cells internalized by the ciliated protozoan *Paramecium primaurelia*. This model system can serve as a tool to test for the protection capabilities against lysosomal enzymes
Methods: Bakers' yeast cells, *S. cerevisae* are conformally coated with auto cross-linking polyelectrolytes. The polymer shells are formed by successive electrostatic deposition of cationic polyamines and reactive polyanions capable of covalently cross-linking with the polycation. Fluorescent labels are used to map the distribution of both polyanions and polycations on the cell surface.
Results: Successful coating of living yeast is reported, while maintaing cell viability.
Conclusions: Encapsulation of living yeast cells allows us to create a model system whereby we can investigate the effects of polymer molecular weight and compositions on cell coating along with cell viability.

P140 (EI0419)

STEM CELL BIONICS: VISION, METHODOLOGY AND FIRST CLINICAL RESULTS

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The physiological mechanisms how stem cells are led into a threedimensional regenerative process are presented as the basis of a bionic concept of stem cell therapy. This platform technology uses the wound as a triggering cofactor for *in situ* and *in vivo* technology as an alternative to *in vitro* stem cell theories and methods. The preparatory process is made possible by tissue specific bioreactors and highly standardized processes. In a diabetic wound the capacity of regeneration is significantly reduced or totally lost. We have developed a technology that topically activates this regeneration potential by endogenous stem cell activation and combines it with an *ad hoc* transplant of cells obtained from the peripheral blood of the patient. This approach intends to mimic the normal wound healing process while the homologous application of blood cells allows paracrine effects of the transplant that improve healing. The scientific rationale and technology focuses on an *in situ* & *in vivo* rather than a conventional *in vitro* use and induces an *in situ* boosting and commitment effect for stem cells that leads to an improved tissue regeneration. The elucidation of the underlying mechanisms, the positive regulatory environment and the safety of the process, the striking preclinical and pilot clinical results do warrant a further development in multicentre clinical trials. Tissue regeneration in skin (burns, diabetic wounds) and bone defects has become a clinical reality. Apart from these areas other tissue applications including cartilage, liver, spinal cord injury, heart valves or trachea are being developed as well. Stem cells are the basis for regeneration and bioreactors are the instruments that make the respective biological technologies available for clinical therapy. Apart from mechanistic studies, preclinical animal trials and early clinical examples are presented.

P141 (EI0410)

***IN VITRO* PERFORMANCE OF K-CARRAGEENAN HYDROGELS COMBINED WITH DIFFERENT TYPES OF CELLS AIMED AT APPLICATIONS IN CARTILAGE REGENERATION**

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Objectives: Injuries of the articular cartilage are one of the most challenging issues of musculoskeletal medicine due to the poor ability of this tissue for repair. Cartilage tissue engineering strategies require the presence of cells and a scaffold material, typically a hydrogel. In this work we have analyzed the *in vitro* performance of k-carrageenan, an ionic hydrogel recently proposed for TE approaches, with encapsulated cells of different types, namely a chondrocytic cell line, primary chondrocytes cells and human adipose stem cells, often proposed for cartilage regeneration strategies.
Methods: The k-Carrageenan hydrogels were produced using an ionotropic gelation method and cells, namely ATDC5 cells, human nasal primary chondrocytes (hNCs) and human adipose stem cells (hASCs), were encapsulated at a density of $5*10^6$cell/mL and cultured for 21 days. The cells viability and proliferation was determined by fluorescence staining, DNA quantification. Chondrogenic differentiation of the different cells encapsulated in the hydrogels was characterized by GAGs quantification, typical histological staining and real time qRT-PCR analysis (Sox9, aggrecan collagen type I, type II and type X).
Results: The biological evaluation of k-carrageenan hydrogel revealed that this polymer enables long-term viability and proliferation of different cells. During 3 weeks of culture, cells encapsulated within the hydrogel developed a cartilage-like extracellular matrix rich in proteoglycans and type II collagen. Cartilage-like ECM deposition and production was found throughout all culturing periods indicating a stable chondrocyte phenotype in encapsulated cells. Nevertheless, encapsulated hASCs exhibit the highest proliferation rates and highest levels of chondrogenic markers expression.
Conclusions: K-carrageenan hydrogels enable the viability and proliferation of different cell types during long-term cell culture. The results obtained indicated the feasibility of using these hydrogels in cartilage tissue engineering approaches due to its ability to support chondrogenic features of different cells types, particularly the hASCs.

P142 (EI0368)

NOVEL GELLAN GUM – HYALURONAN HYDROGEL FORMULATIONS FOR TISSUE ENGINEERING APPLICATIONS

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Objectives: The main goal of this work consisted in the preparation and optimization of glycosaminoglycans (GAGs) hydrogels constituted of hyaluronic acid (HA) and gellan gum (GG) to support cell encapsulation to be used in tissue engineering (TE) purposes.
Methods: Different GG-HA hydrogels formulations, ranging from 1 to 2.5% (m/V) of GG (1 MDa) and from 0.25 to 1% (m/V) of HA (3.5 KDa and/or 2 MDa) were prepared. The *in vitro* enzymatic degradation was evaluated by incubating the hydrogels with hyaluronidase solutions (0, 2.6 and 50 U/mL) for quantification of the resultant fragments using the Morgan-Elson and DNS assays. The mechanical properties of the developed hydrogels were determined by compression tests. The crosslinking efficiency was confirmed by 1H NMR and FTIR-ATR. Finally, the hydrogel morphology was visualized by SEM and micro computed tomography (microCT). The best formulations were selected for further biological assays. Indeed, hASCs were encapsulated in the different hydrogels while the polymerization process occurred. The viability of the encapsulated hASCs was followed along 3, 7 and 14 days after Calcein-AM and Propidium Iodide staining. Cell morphology was visualized after phalloidin staining.
Results: Hydrogels with different mechanical properties were obtained by altering the % (m/V) of the GG-HA formulations. Hydrogels with high percentage of GG were stiffer, while increasing concentrations of HA promoted hydrogel flexibility and higher degradation rates. Moreover, the hydrogels showed an intermediate degradation rate compared to the currently used photocrosslinkable HA-methacrylated hydrogels that rapidly degrade in PBS at 37°C. Furthermore, crosslinking efficiency was confirmed by FTIR-ATR analysis. The hASCs viability was not compromised by the hydrogels.
Conclusions: This work permitted to obtain innovative GG-HA based hydrogels with tuned properties according to the different compositions. More importantly, their capacity to support cell encapsulation makes them very appealing for different TE applications.

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P143 (EI0367)

CRYOPRESERVATION OF ENGINEERED TISSUES: CT-VISUALIZATION OF CPA DIFFUSION IN SCAFFOLDS

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Objectives: Effective long-term storage of native and engineered tissues poses a specific challenge for biomedical applications. The diffusion of cryoprotective agents (CPA) into tissue is one of the major hurdles for successful cryopreservation. In 3-D engineered scaffolds CPAs like dimethyl sulfoxide (DMSO) should be homogeneously distributed to protect cells from freezing damages. A local excess of CPA in the construct will damage the cells due to the general toxic effects of CPAs, whereas insufficient CPA concentrations will lead to cryopreservation damage. This study was performed to measure and visualize the effective diffusion of DMSO within engineered collagen scaffolds using computer tomography (CT).

Methods: Collagen scaffolds with a pore size of 100µm (dimension: 30x30x10mm^3) and a porosity of 98% were self-manufactured [2]. Unseeded scaffolds and with 3T3-NIH cells seeded scaffolds were stored in PBS and respectively DMEM. The scaffolds were transferred directly in 10% (v/v) DMSO. Computer tomographic images were acquired immediately every 1.5 minutes over a period of 3 hours. Grey scale values that were determined from the images were converted in DMSO concentration and indicate CPA dispersion within the tissue. The DMSO loading process of the scaffold could thus be measured and visualized in real time.

Results and Conclusions: The study showed that incubation times of more than 3h are required to achieve homogenous CPA distribution in unseeded collagen scaffolds of this size [3]. A model, established from experimental data, allows determining adequate exposure times for different construct sizes.

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P144 (EI0360)

USING HUMAN ADIPOSE-DERIVED STEM CELL ON BACTERIAL CELLULOSE MEMBRANES FOR THE PRODUCTION OF NEW SKIN SUBSTITUTES

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Objectives: The study of the dynamic behavior and adhesion between cells and substrates in skin tissue engineering is of major importance to predict the final biological properties of tissue implants. The adhesion of cells on the substrate influences morphology, proliferation and cellular viability. In this work, adhesion, proliferation and viability of s on bacterial cellulose (BC) membranes were evaluated *in vitro*. The objective of this work was to demonstrate a method for obtaining new skin substitutes using Human Adipose-derived Stem cell and keratinocytes on bacterial cellulose membranes

Methods: Biological effects of these bacterial cellulose membranes were evaluated by transmission electron microscopy (SEM), confocal microscopy, protein expression, viability and cytokine assays.

Results: All these results suggest that bacterial cellulose membrane is a potential candidate for dermal equivalent with enhanced biostability and good biocompatibility.

Conclusions: These results indicate that hASC seeded onto Bacterial cellulose membranes allowed cell adhesion, growth, proliferation and viability. We believe that the present model for human skin reconstructed *in vitro* has excellent applicability in relation to laboratory studies and good prospects for clinical use.

P145 (EI0359)

NEW APPROACH TO HUMAN ADIPOSE STEM CELL SEEDED ON PHB-HV SCAFFOLDS FOR BONE TISSUE ENGINEERING APPLICATIONS

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Objectives: Tissue engineering emerges as a field of regenerative medicine that offers a strategy to circumvent the major problems related with regenerative and therapeutic procedures including bone grafting. Therefore, our group have examined the possibility of using a pool of allogeneic human serum (alloHS) as supplement of the osteogenic medium to evaluate the growth patterns and osteogenic differentiation of hASC on poly-3-hydroxybutyrate-co-3-hydroxyvalerate (PHB-HV) scaffolds, aimed to be used in bone tissue engineering.

Methods: In this study, hASC were obtained from lipoaspirates and expanded in medium containing *Dulbecco's modified Eagle's medium/F12* supplemented with 10% alloHS. Immunophenotypic characterization with flow cytometry was performed. The PHB-HV scaffolds used were developed by freeze-drying technique and characterized by SEM and µCT. The cells were seeded onto the PHB-HV scaffolds under static condition and cultured by different times in non-osteogenic and osteogenic medium. Through *in vitro* assays, the viability of these cells was determined by MTS assay. Cell adhesion and morphology were observed by SEM. And the osteogenic potential of these hASC were assessed by alkaline phosphatase quantification, mineralization content and expression of bone markers.

Results: The scaffolds showed a good porosity and interconnectivity allowing adhesion and proliferation of hASC. The cells cultured in DMEM 10% alloHS showed the immunophenotype characteristic for mesenchymal stem cell, high viability and were capable of differentiate into the osteogenic lineage.

Conclusions: All these results indicate that hASC seeded onto PHB-HV scaffolds and cultured in alloHS medium may be a suitable strategy to induce bone formation. And the data presented here are important for bone tissue engineering.

P146 (EI0357)

DENDRITIC CELLS AS RELEVANT TOOLS TO PREDICT THE OUTCOME OF TISSUE ENGINEERING CONSTRUCTS

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Objectives: Within TE constructs, cells represent adjuvants to the host immune system, acting through the maturation of dendritic cells (DCs), leading to increased co-stimulatory and MHC molecules expression, cytokine secretion and allo-stimulatory capacity. Starch and poly-caprolactone (SPCL) scaffolds have shown to support human adipose-derived mesenchymal stem cells (hASCs) growth and differentiation. The aim of this work was to evaluate the interaction of hASCs within SPCL scaffolds with DCs, gathering further knowledge on the immunomodulatory potential of these constructs.

Methods: SPCL scaffolds, obtained by wet spinning methodology (SPCL-WS), seeded with hASCs were cultured in standard cell culture conditions for 48h prior to contact directly with DCs for further 24h, 48h and 72h. DCs were differentiated from the mononuclear fraction of human peripheral blood cultured with IL-4 and GM-CSF for 48h. The maturate and activated phenotype of DCs was evaluated by flow cytometry and RT-PCR before and after culture with the TE constructs, and compared with mature DCs, expressing CD80, CD83, CD86 and MHC class-II and lacking CD14 after incubation with LPS.

Results: The findings showed that DCs maintain their immature status at days 3 and 7 days after replating, demonstrating low expression of CD80 and CD83. As replating procedures were shown to be a critical step in the routine evaluation of TE constructs, this is a critical issue to address. Although DCs do not express the maturation markers, their genetic profile showed the presence of CD80, CD83, CD86 and CD1a, indicating that cells are capable of expressing those markers after adequate stimuli.

Conclusions: These TE constructs (SPCL-WS scaffolds and hASCs) showed the inability to induce allogenic DCs activation after direct contact culture. This supports the conclusion that the assembled TE constructs will be well tolerated by the host in an allogenic approach and might further indicate their immunomodulatory potential.

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P147 (EI0353)

MICROENCAPSULATION OF CELLS IN ALGINATE BEADS USING HIGH VOLTAGE ELECTROSPRAYING

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Objectives: Cell transplantation is currently being investigated for the treatment of various disorders such as liver failure, diabetes etc. Since the source of these cells is non-autologous, immunogenicity is a major concern. To get around this problem, one can encapsulate the cells in a hydrogel matrix such as alginate beads. Encapsulation of cells can be also used to protect the cells during transportation, cryopreservation etc. Here we describe a method for production of such cell-encapsulated alginate beads using high voltage to control the size of the beads.
Methods: NIH-3T3 cells were incubated in a suspension of 1.5% Alginate solution for 2 hours. The solution was drawn into a syringe, whose nozzle was connected to a high voltage source. The flow rate was controlled using a precision pump. Drops formed via electrospraying were collected in a cross-linking bath of $CaCl_2$ or $BaCl_2$ which was kept grounded. The resulting beads were washed with PBS before determining post-process cell survivability using fluorescence microscopy.
Results: The typical size of the beads ranged between 800µm and 1.3mm, which could be controlled by changing the process parameters such as flow rate and the applied voltage. The number of cells encapsulated per bead could be controlled by changing the initial cell concentration in suspension. Qualitative analysis using fluorescence imaging showed that cells survived the process.
Conclusions: Here, we describe a method to produce cell-encapsulated alginate beads using high voltage. Compared to gravity-driven systems, this technique offers more control over the size of the beads. Further experiments are currently being planned to study long-term effects of this process, as well as the possibility to encapsulate other cell types.
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P148 (EI0371)

THREE-DIMENSIONAL (3D) CAPILLARY-LIKE STRUCTURE FORMATION WITH THE HELP OF BIOFUNCTIONALIZED MICROFIBERS

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Objectives: Cell-providing vessels are indispensable throughout an engineered tissue with more than 100-200µm of thickness. To control vascular network formation *in vitro*, fibers can be used to position vessel-forming endothelial cells within a 3D matrix. This support can then lead to capillary-like structure formation next to the fibres, supported by growth factors. With this method, a local-controlled capillarization can be achieved.
Methods: Biofunctionalization of poly-DL-lactide acid (PDLLA) fibres is done by aminofunctionalization and covalent binding of RGD peptides, vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) via a hexamethylene diisocyanate (HDI) spacer. RGD is an amino acid sequence supporting cell attachment, whereas VEGF and bFGF promote capillary-like structure formation. After positioning of the biofunctionalized fibers in a moulding form, human umbilical artery smooth muscle cells (HUASMCs) and human umbilical vein endothelial cells (HUVECs) were seeded on the fibers. Fibrin gels were moulded directly or 4 days after cell seeding on the fibers inside the wells. After 9 days of co-culture, the gels were fixed and immunostained with CD31. The formation and quantification of capillary-like structures in the 3D fibrin matrix was done using two-photon microscopy and ImagePro Analyzer software.
Results: Capillary-like networks formed mainly on the lowest plane of the fibrin gel, but also near the fibers. When the fibrin moulding was done 4 days after cell seeding, capillary-like structures formed directly next to the functionalized microfibers. The co-cultivation of HUASMCs seem to sufficiently support HUVECs by forming capillary-like structures.
Conclusions: Vascular network formation can be realised and controlled next to biofunctionalized fibers inside a 3D fibrin matrix. Two-photon microscopy helps to visualize and quantify the capillary-like structures. Ideally, degradable fibers should be used in the future while the intact capillary-like network remains inside the fibrin matrix. Furthermore, mechanical stimuli can help building up tubule-like structures with lumen.

P149 (EI0350)

ELECTRO HYDRO DYNAMIC (EHD) ENCAPSULATION OF CELLS IN ALGINATE BASED HYDROGELS

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Objectives: The possibility of cells encapsulation in a polymeric bead with proper characteristics offer many advantages for clinical applications in tissue repair since it is possible to tailor these beads for a controlled release of entrapped cells on a repair site. The objective of this study is to evaluate the possibility to use an electro hydro dynamic (EHD) system as a mean to encapsulate living mammalian cells in alginate based hydro-gels.
Methods: Capsules are manufactured starting from a tailored alginate-based liquid solution containing mammalian cells, drops are formed through a needle by a EHD system and these drops are immediately cross-linked in a calcium based solution. The effect of the process on cell viability is assessed and confocal microscopy images will be presented.
Results: EHD seems a versatile technique to encapsulate living cells. It allows the creation with high throughput of round beads up to 100um in which cells remain viable starting from solutions characterized by a wide range of viscosity. The viscosity of the solution has an impact on the ability of forming and detaching drops from the EHD needle and as such on their final geometry, while the cross-linker properties determine the morphological characteristics of the final capsules.
Conclusions: If the encapsulation of a given cell line is desired, it is important to know what are the ranges of the parameters that characterized the encapsulation process which can be used in order to obtain beads with desired geometry containing viable cells for a specific application.

P150 (EI0375)

COMBINATORIAL CELL-POROUS SCAFFOLDS INTERACTIONS STUDY AND SCAFFOLD PHYSICOCHEMICAL CHARACTERIZATION IN AN INNOVATIVE BIOINSPIRED HIGH-THROUGHPUT PLATFORM

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Objectives: Superhydrophobic surfaces patterned with size-controlled hydrophilic spots are proposed for the production of porous scaffolds arrays for combinatorial biomaterial-cells interaction studies. This approach allows for the open and easy access to scaffolds for further follow-ups aimed at high-throughput screening, when compared with the other scaffold array production techniques using commercial well plates or soft lithography-produced wells. Mechanical and morphological characterization along with cell study using chitosan/alginate mixtures with different concentrations of fibronectin were the proposed objectives.
Methods: Polystyrene superhydrophobic surfaces were prepared by a phase-separation method. Aluminum hollow masks were used to pattern 4mm^2 hydrophilic spots by UV/O_3 exposure. Chitosan/alginate mixtures in 100:0, 75:25 and 50:50 proportions along with 3 different concentrations of fibronectin were placed on the spots. L929 and MC3T3 cell lines were used for cell viability (by MTS test) and cell number (by dsDNA quantification) study. Future work aims to the characterization of the structures on the chip by dynamic mechanical analysis and µCT.
Results: Cell culture results were mostly favorable for 50:50 chitosan/alginate proportion for both cell types. In this condition, fibronectin in the highest concentration enhanced cell viability using L929 cells, while in MC3T3 cells the use of all concentrations of fibronectin showed increased adhered viable cells compared to the non-adsorbed fibronectin condition. Regarding cell number, generally, the presence of fibronectin affected MC3T3 cell line favorably, as well as L929 in the 100:0 polymer ratio condition. Overall, a tendency for enhanced cell response can be seen in the highest concentration of fibronectin.
Conclusions: A biochip for combinatorial analysis of biomaterial-cells interactions based in the extreme wettability of patterned spots in superhydrophobic

surfaces was developed for the study of natural polymer porous scaffolds. Tendencies could be seen in cell response of two cell lines according to polymer and adsorbed protein gradients.

P151 (EI0343)

L-PROLINE AND ECTOINE STABILIZE PROTEINS FROM DENATURATION

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Objectives: Compatible solutes such as ectoine, hydroxyectoine and L-proline have been reported to be able to stabilize lipids and proteins in cell membranes. Recently it has been found that L-proline and ectoine could be applied in cryopreservation of human stem cells as cryoprotective agents (CPA). Thus, the conventional CPA dimethyl sulfoxide, which is cell toxic, could be replaced. The working mechanism of compatible solutes was explained as "preferential exclusion". In this study we have studied the effects of L-proline and ectoine on the denaturation of model protein bovine RNase A with differential scanning calorimeter (DSC).
Methods: Nano DSC from TA Instruments was used in this study. Bovine RNase A, which is well studied, was used as model protein. Protein solution was mixed with different concentrations of L-proline and ectoine (between 10mM and 4M), and concentration of RNase A was kept at 2mg/mL. Samples were examined with DSC in a temperature range from 20°C to 100°C. Protein denaturation onset temperature, melting temperature, calorimetric enthalpy, change of the specific heat capacity and the fraction of the denatured protein were determined by analyzing the DSC data curves.
Results: DSC results showed that L-proline and ectoine elevate melting temperature with a positive relationship with their concentration, in the presence of 2M L-proline, protein melting temperature was 1.5K higher than that without L-proline. Melting enthalpy of bovine RNase A was also increased, small concentrations of L-proline (10mM to 100mM) could lead to the maximum elevation, however ectoine needs relative higher concentration (500mM). In the presence of 2M L-proline and 4M ectoine, protein denaturation was highly. The results are in agreement with literature data.
Conclusions: Compatible solutes L-proline and ectoine are able to stabilize bovine RNase A, L-proline needs relatively lower concentrations as compared to ectoine.

P152 (EI0382)

DENTAL PULP STEM CELLS AND NANOFIBROUS SCAFFOLDS FOR APPLICATION IN TISSUE ENGINEERING

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Objectives: Organs of the human body are nanostructures and cells in the body are accustomed to interact with materials that have nano-structured features and hence the development of nanofibers by using the technique of electrospinning is having a new momentum. Smart engineered scaffolds loaded with stem cells can differentiate into specific cell lineages for effective tissue regeneration. Regarding the importance of stem cells and nanofibrous scaffolds, the aim of this study is the investigation of suitability of Poly (ε-caprolactone) PCL/gelatin nanofibrous scaffolds for dental pulp stem cells attachment and proliferation.
Methods: The polymer solution with concentration of 6wt% of PCl/gelatin (70:30) was electrospun at high voltage of 12 kV. The morphology, mechanical properties and hydrophilicity of nanofibers were studied using scanning electron microscopy (SEM), uniaxial testing and contact angle measurement respectively. Dental pulp stem cells were seeded on PCL/gelatin nanofibrous scaffolds and tissue culture plate (TCP) as control. MTS assay was used for investigation of proliferation of stem cells on nanofibrous scaffolds after 3 and 5 days of cell seeding. The morphology of cells on nanofibrous scaffolds was observed after 5 days of cell seeding using SEM.
Results: From SEM images of nanofibers, fiber diameter was estimated to be 189±56 and mechanical testing and contact angle measurement revealed the suitability of PCL/gelatin nanofibrous scaffolds in sense of tensile strength and hydrophilicity. MTS assay revealed the proliferation of stem cells on PCL/gelatin nanofibrous scaffolds. The cell proliferation on nanofibers was found to be higher than that on TCP after 5 day of cell seeding. SEM micrographs of cells on PCL/gelatin nanofibers also showed the attachment and spreading of cells on nanofibrous scaffolds.
Conclusions: In summary, our results showed the suitability of PCL/gelatin (70:30) nanofibrous scaffolds as a substrate for dental pulp stem cells.

P154 (EI0385)

ARTIFICIAL LET-7G DOWNREGULATION FOR INDUCTION OF LIVER REGENERATION – INITIAL RESULTS WITH HEPG2 CELLS AND PRIMARY HUMAN HEPATOCYTES

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Objectives: It is already known that global downregulation of microRNA family let-7 members plays a crucial role in carcinogenesis due to their function as growth suppressor. Recent data indicate that temporal let-7 downregulation is involved in regulation of liver regeneration. The aim of this study was to investigate if artificial downregulation of let-7g could be used to induce upregulation of proliferation-inducing genes in tumor cells and quiescent primary human hepatocytes (PHH).
Methods: HepG2 cells and PHH were used for *in vitro* studies. Antisense oligonucleotides against hsa-let-7g were inserted by liposom transfection for 24 hours. Effects of transfection were investigated using real-time Polymerase Chain Reaction. Protein expression of putative targets of let-7 family members (Cyclin D1 and c-Myc) was investigated by Western Blot analysis.
Results: Endogenous expression of let-7g was lower in HepG2 cells compared to PHH. Transfection of HepG2 and PHH with antisense-let-7g caused significant decrease of endogenous let-7g expression. We reached a knockdown ratio of nearly 100% in cultivated HepG2 and in PHH. While we did not observe alterations in protein expression of c-Myc and Cyclin D1 in HepG2-cells, we observed significant upregulation in PHH following artificial let-7g knockdown.
Conclusions: Our initial results show that a depletion of let-7g seems to have an increasing effect on cell cycle modulating proteins such as c-myc and Cyclin D1 in cultivated PHH. The downregulation of let-7g could be a potential opportunity of miRNAs based therapeutical strategies for induction of liver regeneration.

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P155 (EI0331)

RATIONAL DESIGN FOR IRRIGATION DRIPPING TRIPLED PERFUSION BIOREACTOR

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Objectives: The post-printing tissue maturation requires development of new type of perfusion bioreactor. The rational design behind such bioreactor, especially the level of porosity and distance between minitubes must be based on systematic mathematical modeling and computer simulation of interstitial flow. The proposed novel irrigation dripping tripled perfusion bioreactor will enable to 'buy' time until the 'built in' intraorgan branched vascular system will mature enough for initiation of intravascular perfusion.
Methods: Fick's formula has been used to estimation diffusion coefficient for oxygen and small molecular weight tracers such as dextran or bovine serum albumin. It was assumed that hydrogel has isotropic properties. Mathematical model for diffusion and diffusion enhanced by convection has been developed. Computer simulation of diffusion gradient has been explored using color coding of trace molecule concentration.
Results: Mathematical modeling has shown that with increasing minitube porosity the diffusion distance will also increase. The diffusion with additional convection will increase distance between porous minitubes. Thus, rational design of irrigation dripping tripled perfusion bioreactor must have combination of proper level of minitube porosity and maximal possible and biologically acceptable distance between minitubes. Computer simulation of interstitial flow and estimated parameters for rational design of perfusion bioreactor have been confirmed experimentally by analysis of diffusion of tracer molecules from porous needle placed in different type of hydrogels. It has been also demonstrated that during tissue maturation the diffusion and interstitial flow in bioprinted tissue construct will be gradually reduced.
Conclusions: Mathematical modeling and computer simulation have been used to estimate proper design parameters. Thus, it has been demonstrated that with implementation of rational design based on mathematical modeling and computer simulation development of irrigation dripping tripled perfusion bioreactor is a realistic goal.

P156 (EI0329)

SCALABLE BIOFABRICATION OF UNIFORMED SIZED TISSUE SPHEROIDS FOR ORGAN PRINTING

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Objectives: Organ printing is a rapidly emerging technology based on layer-by-layer additive robotic biofabrication of 3D tissue and organ constructs using self-assembling tissue spheroids as building blocks. In order to print human organ constructs it will be necessary to fabricate millions of tissue spheroids. In this context the development of methods for scalable biofabrication of uniformly sized tissue spheroids is essential for tissue spheroid-based bioprinting of large size tissue and organ constructs.
Methods: Two new molds were designed to enable generation of microrecessions in non-adhesive agarose hydrogel. The microrecessions were seeded with human adipose tissue-derived stem cells using manual and automated pipetting approach. Size redistribution and shape of biofabricated tissue spheroids have been estimated.
Results: After 48 hours of incubation, tissue spheroids formed at the bottom of each microrecession. To assess the quality of constructs generated using this technology, six hundred tissue spheroids made by this method were compared with six hundred spheroids generated by the conventional hanging drop method. These analyses showed that tissue spheroids fabricated by the micromolded method are more uniform in diameter and shape than by conventional hanging drop methods. The main advantage of using new molds with optimized geometry is the capacity to use robotic dispenser and thus automate and scale up process of more uniform tissue spheroids biofabrication.
Conclusions: Thus, the use of micromolded recessions in a non-adhesive hydrogel, combined with automated cell seeding, is a reliable method for scalable robotic biofabrication of uniform-sized tissue spheroids. The tissue spheroids of standard size could be used in drug discovery and toxicity assays, in direct differentiation of stem cells into specific minitissues (for example cartilage or bones) and most importantly for robotic bioprinting of complex human organs.

P157 (EI0321)

INFLUENCE OF POLYLACTIDE FIBERS WITH SPIN FINISHES ON THE TOXICITY *IN VITRO*

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Objectives: Biodegradable fibers with controlled properties may meet the requirements for medical applications. Poly(lactic acid) (PLA) is a biodegradable linear aliphatic thermoplastic polyester. PLA fibers with five type spin finishes were prepared and assayed for *in vitro* cytotoxic activities.
Methods: The PLA fibers were prepared by a two-step melt-spinning process. The PLA Polymer 6201D, fiber grade with nominal MFI=15-30 g/10min, a NatureWorks LLC product was used. PLA fibers were coated with 5 types of spin finishes: PLA 24 with 2.4% of Glicerol Ph Eur, PLA 25 – 0.40% of Lurol PL 801, PLA 26-0.61% of Stantex 6457, PLA 27-0.36% of Lurol PT-L216, PLA28-0.62% of Estesol PF 790. The fibers with linear density 2.2 – 4.8 dtex, tenacity 35 – 39 cN/tex, elongation ~50% were obtained. To determine if they can affect cells, line cultures L929 (ATCC CCL1) was used. The cells ($2x10^6$ cells/mL) were incubated with fibers for 24h, 48h and 72h (37°C, 5% CO_2). Cell growth, morphology and viability were determined.
Results: After 72h incubation, the level of cytotoxicity of PLA 24 fibers was 2 (% dead-38), PLA 25 - 3 (% dead-100), PLA 27 - 3 (% dead-100), PLA 28- 0 (% dead-99), control fenol-3 (% dead-94), L929-0 (% dead-3).
Conclusions: Fibroblast cultures after contact with the four of PLA fibres showed cytotoxity effects. The cells were dead with hanged morphologie and lower proliferation. The result of the testing of PLA fibers with Estesol spin finish did not show any cytotoxicity effects and may be promising candidate for medical applications.
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P158 (EI0316)

PRP INFLUENCE ON THE REGENERATION OF REPRODUCTIVE SYSTEM IN THE EXPERIMENT

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Objectives: To analyse the medical influence of platelet rich plasma (PRP) on the reproductive function of mice in experimental toxic influence condition.
Methods: ICR mice at the age of 4-5 months were used. For modeling pathology of reproductive system 2 intraperitoneal injection of Adriblastin in summary dose of 2mg/kg were done with an interval of one week. The mice were divided into two groups: the 1st is the control group; in the mice of the 2nd group we have injected PRP in scrotum on the second day after last injection of Adriblastin. PRP was injected three times with an interval of 2 weeks. Pathomorphologic analysis of sections was done on the 4 and 6 weeks after PRP injection.
Results: On the 4th week the extension of convoluted channels, significant decreased amount of spermatogonia and other cell types of spermatogenic epithelium can be noticed in the 1st group in comparison with intact animals. Two weeks later interstitium and basal membrane are extended and hyperchromic, due to edema and inflammation, the amount of spermatozoa is still reduced. In the 2nd group visible increase of all cell types of spermatogenic epithelium can be noticed after 4 weeks post-treatment. Spermatogonia recovery is an evidence of high healing effect of PRP, because Adriblastin influence negatively exactly on these germinative cells of spermatogenic epithelium. Interstitium and basal membrane are still extended, but they are in better morphologic condition as they are in the 1st group in the meantime. On the 6th week convoluted channels are partially extended, but they are reverting to the norm. The state of organ is recovering.
Conclusions: PRP makes good results in treating toxic defeat of the reproductive system.

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P159 (EI0315)

THE DEGRADATION OF PORCINE ENDOGENOUS RETROVIRUSES DNA IN ACELLULAR PORCINE HEART VALVE SCAFFOLDS FIXED WITH DIFFERENT LOW TOXIC AGENTS

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Objectives: The application of tissue engineered scaffolds based on porcine heart valves is connected with danger of porcine endogenous retroviruses (PERVs) transmission because acellularization process might not remove all PERVs DNA from tissue. Chemical fixation with glutardaldehyde induces complete degradation of PERVs genetic material in porcine tissue, but this compound is too toxic for cells which will be seeded on scaffolds. Therefore the purpose of the present study was to investigate how chemical fixation with low toxic agents, i.e. two different derivatives of flavonoids – DF1 and DF2, and genipin influence on the PERVs DNA existence in acellular porcine heart valve scaffolds.
Methods: Porcine pulmonary and aortic valves were acellularized using Trypsin/EDTA and sodium sulphate. Acellular tissues were treated with 1) flavonoid derivative DF1, 2) flavonoid derivative DF2 or 3) genipin. The fixation was carried out at 20°C for 3 days. Genomic DNA was isolated from native, acellular and acellular fixed tissues by means of salting out extraction method. Quantification of PERV-A, PERV-B and PERV-C DNA was performed by real time Q-PCR technique. Native and acellular valves were used as a control.
Results: All subtypes of PERVs were detected in native porcine heart valves. Reduction of copies number of PERV-A, PERV-B and PERV-C DNA was observed in acellular porcine valves as well as in acellular valves fixed with flavonoid derivative DF1 and with genipin. PERVs DNA was completely degradated only in acellular porcine heart valves fixed with flavonoid derivative DF2.
Conclusions: Our results demonstrated that fixation of acellular porcine valves with flavonoid derivative DF2 causes completely degradation of PERVs DNA in tissues, thus the acellular porcine heart valve scaffolds fixed with low toxic agents can be used for transplantation without risk of PERVs transmission.

P160 (EI0407)

INTERACTION OF CHONDROCYTES WITH ELECTROSPUN POLYMER SCAFFOLDS DEPENDING ON THE FIBER ORIENTATION

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Objectives: Biocompatible polymer-based scaffolds with a tailaroble degradation rate and a predefined structure might provide an approach to improve cartilage repair, which is limited by the poor intrinsic healing capacity of cartilage. The aim of this study was to explore whether electro-spun polymer scaffolds with different fiber orientation could influence the growth of primary articular chondrocytes.
Methods: Electro-spun scaffolds with aligned and random fiber orientation were prepared from two degradable polymers: poly(ether)ester urethane (PDC) and poly(*p*-dioxanone) (PPDO) as well as polyetherimide (PEI) as a reference polymer, which is not intended to degrade. PDC was selected as a candidate material showing an almost linear mass loss in hydrolytic and enzymatic *in vitro* degradation experiments. Electro-spinning was conducted at ambient temperature using hexafluoro-2-propanol (HFP) as solvent for PDC and PPDO, while PEI was processed from dimethylacetamide (DMAc) solution. The electro-spun structures exhibited an average deposit thickness of 80±20µm with a single fibre diameter around 2-3µm. Primary porcine articular chondrocytes were seeded on the ethylene oxide sterilized scaffolds and analyzed for vitality, ultrastructure and type II collagen expression.
Results: Satisfactory numbers of vital chondrocytes could be detected on all electro-spun scaffolds, which were able to produce the cartilage-specific protein type II collagen. An almost flattened cell shape of the chondrocytes was observed on scaffolds with random fiber orientation, while on scaffolds with aligned fibers the chondrocytes exhibited a spherically cell shape and penetrated into the scaffold pores between the parallel fibers. Surprisingly, it was found that the chondrocytes did not align along the fiber direction.
Conclusions: Chondrocytes were able to grow on all polymer scaffolds tested and expressed the differentiation marker type II collagen. Cell morphology differed depending on the fiber orientation within the scaffolds.

P161 (EI0291)

IONIC LIQUIDS IN NOVEL PROCESSING WAYS TO OBTAIN CHITOSAN/SILK FIBROIN HYDROGELS FOR SKIN TISSUE ENGINEERING

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Objectives: The main goal of this work renders the application of green chemistry principles, namely the use of ionic liquids (ILs) and biorenewable sources, such as chitosan (CHT) and silk fibroin (SF), to newly process hydrogel-based constructs. The combination of this polysaccharide and protein may mimic the naturally occurring tissue environment. Although the solubilization of both materials in ILs has been studied individually, this work reports, for the first time, the use of ILs as a common solvent, for blended CHT/SF hydrogels production. These systems offer the advantage of being homogeneous and of presenting easy and short dissolution time of both biomacromolecules. Moreover, the intrinsic properties of these biopolymers are expected to accelerate the regeneration of chronic skin wounds.
Methods: Hydrogels were obtained by dissolving CHT and SF in 1-butyl-3-methylimidazolium acetate, [bmim][Ac] (4wt%) at different ratios. The systems were gelified and ILs removal was performed by soxhlet extraction with ethanol. The effect of the chitosan source and CHT/SF ratio on consistency, crystallinity, protein adsorption and mechanical properties was evaluated. Moreover, the ability of the developed materials to support adhesion and proliferation of human dermal fibroblasts (hDFb) was assessed up to 21 days of culture.
Results: The findings suggest that [bmim][Ac] allowed the production of CTH/SF hydrogels with a soft and rubbery consistency, microporous surface, good protein adsorption and viscoelastic behavior. Additionally, *in vitro* biological performance revealed a positive influence over adhesion, viability and proliferation of hDFb.
Conclusions: The use of [bmim][Ac] as a common solvent provided a versatile approach to obtain CHT/SF hydrogels with interesting properties and with potential to sustain dermal fibroblasts outgrowth. This work constitutes a strong basis for future healing studies of chronic skin wounds.

P162 (EI0279)

MICROVESICLES DERIVED FROM HUMAN ADULT MESENCHYMAL STEM CELLS PROTECT AGAINST ISCHEMIA-REPERFUSION-INDUCED ACUTE AND CRHONIC KIDNEY INJURY

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Objectives: Several studies demonstrated that mesenchymal stem cells (MSCs) reverses acute kidney injury (AKI) in different experimental models by a paracrine mechanisms rather then by MSC transdifferentiation. We recently demonstrated that microvesicles (MVs) released from MSCs may account for this paracrine mechanism by an horizontal transfer of mRNA and microRNA.
Methods: MVs were purified from MSC supernatants and were injected intravenously in rats (30mg/rat) immediately after monolateral nephrectomy and renal artery and vein occlusion for 45 minutes. To evaluate the MV effects on AKI induce by IRI, the animals were divided into different groups: normal rats (n=4), sham operated rats (n=6), IRI rats (n=6), IRI+MV (n=6), IRI+RNase-MV (n=6) and all animals were sacrificed at day 2 after operation. To evaluate the CKD induced by IRI, the rats were divided into different groups: sham operated rats (n=6), IRI rats (n=6), IRI+MV (n=6) and all animal were sacrificed 6 months after the operation.
Results: We found that a single administration of MVs, immediately after induction of ischemia-reperfusion injury, protects rats from AKI by inhibiting apoptosis and stimulating tubular epithelial cell proliferation. The MVs also significantly reduced the impairment of renal function induced by ischemia reperfusion injury. Pre-treatment of MVs with RNase to inactivate their RNA cargo, abrogated these protective effects. Moreover, MVs protected from chronic kidney disease observed at 6 months in control rats with ischemia reperfusion injury.
Conclusions: MVs released from MSCs protect from ischemia reperfusion induced AKI and chronic renal injury, suggesting that MVs could be exploited as a potential new therapeutic approach.

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P163 (EI0278)

FAST DYNAMIC MRI MONITORING DURING LIVER CELL TRANSPLANTATION TO THE SPLEEN IN A PORCINE MODEL

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Objectives: Liver cell transplantation (LCT) is a promising approach for the treatment of metabolic liver disorders. However, a method for non-invasive monitoring during LCT is not available clinically, and thus little is known about the processes during and following LCT. The aim of this study was to investigate the feasibility of fast dynamic magnetic resonance imaging (MRI) monitoring during liver cell infusion to the spleen, which is considered an ectopic implantation site for LCT.
Methods: Male porcine liver cells were labeled with micron-sized iron oxide particles (MPIO) and infused to the spleen of female fully-grown pigs (n=5) through a catheter placed in the lineal artery. MRI monitoring was performed using a conventional 3.0 Tesla MR scanner. Initially, T1- and T2-weighted pulse sequences were tested for the detection of MPIO-labeled cells in the spleen. Thereafter, fast dynamic MRI was performed during cell infusion. MR findings were verified by histological and immunohistological examination.
Results: Images from static MRI (repetition time / echo time: 2,500/105.2ms) showed significantly lower signal intensity and signal-to-noise ratio after cell infusion compared to pretransplant images. T2-weighted fast dynamic MRI enabled visualization of continuous signal decrease of the spleen during cell infusion. T1-weighted sequences did not show signal decrease at the same time. When cells were infused systematically, no signal changes in the spleen were observed.
Conclusions: This study shows that fast dynamic MRI can enable non-invasive visualization of liver cell distribution in the spleen and verification of the success of cell delivery. This approach could be useful for preclinical studies and for quality control of LCT in the clinical setting.

P164 (EI0267)

SILICON GRAFTED COLLAGEN AS A SCAFFOLD FOR REPAIRING TYMPANIC MEMBRANE PERFORATIONS: *IN VITRO* AND *IN VIVO* ASSAYS

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Objectives: Chronic tympanic membrane perforations are treated by myringoplasty operation. Although multiple autologous grafts, allografts, and synthetic graft materials have been used over the years, no single graft material is superior for repairing all perforation types. Recently, our group have observed remarkable properties of collagen grafted polydimethyl siloxane (Col-g-PDMS) therefore, in this study Col-g-PDMS potential was assayed as tympanic membrane patch.
Methods: Collagen was grafted onto substrate via a two-step plasma treatment. Then both the biocompatibility of the modified films and cell behavior on the surface of these films were investigated by *in vitro* tests using mouse fibroblast cell (L929), and 12 patients underwent Col-g-PDMS myringoplasty in order to investigate its position *in vivo*.
Results: It was observed that collagen immobilized surfaces showed significant cell adhesion and growth in comparison with the unmodified samples. The overall efficacy of Col-g-PDMS myringoplasty was 75% with total closure, and reduction in size of perforation in 25% (after one attempt). In each of the remaining three, there was a disturbing cause leading to failure.
Conclusions: The Col-g-PDMS grafts were found to be feasible for tympanic membrane perforations.

P165 (EI0265)

INFLUENCE OF CONDITIONED MEDIA IN CARTILAGE-LIKE TISSUE PRODUCTION IN CO-CULTURES OF ARTICULAR CHONDROCYTES AND WHARTON´S JELLY-DERIVED STEM CELLS

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Objectives: Soluble factors released by chondrocytes have been shown to influence stem cells differentiation onto the chondrogenic lineage. The use of conditioned medium obtained from chondrocytes for stimulating stem cells chondrogenic differentiation may be a very interesting alternative for the clinical application of these cells. Therefore, we tested the influence of conditioned medium obtained from articular chondrocytes cultures to determine its influence on co-cultures of human Wharton's jelly MSCs (hWJSCs).
Methods: In the present work, direct and indirect co-cultures (using conditioned medium obtained from a culture of human articular chondrocytes) with hWJSCs were established. Cells were isolated from human samples collected at the local hospital, under donors' informed consent. The co-cultures were performed in 3D scaffolds, composed by a blend of 50/50 chitosan and poly (butylene succinate) – CPBS. Co-cultures were maintained during 28 days.
Results: Wharton's jelly MSCs were able to undergo chondrogenic differentiation. By the end of the experiment co-cultures showed glycosaminoglycans (GAGs) accumulation and specific cartilage-related genes expression, for both types of co-cultures. Indirect co-cultures results show that the chondrogenic differentiation and cartilage ECM formation is enhanced compared to the direct ones.
Conclusions: The use of conditioned medium obtained from articular chondrocytes induced WJSCs chondrogenic differentiation and ECM formation. The obtained results showed that this new strategy is very interesting and should be further explored for clinical applications.

P166 (EI0146)

THE SYNERGISTIC EFFECTS OF STARLING FLOWS AND A DISTRIBUTED AND DELOCALIZED NUTRIENT SOURCE ON BONE MARROW STROMAL CELL CULTURE IN HOLLOW FIBRE MEMBRANE BIOREACTORS

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Objectives: The development of large dimensions bone tissue engineered constructs is limited by the difficult delivery of nutrients to cells situated in the inner part of them. In this study, hollow fibre membrane bioreactors (HFMB) were used to allow a distributed and delocalized nutrients supply in 3D cm-scale constructs. Low to high spontaneus convective flows effect on cell distribution, proliferation and collagen deposition were investigated.
Methods: HFMB where seeded with $4.5x10^6$ BMSC cells/mL. Cells were fed with culture medium from the membranes lumen in a recirculation modality for 12 days. Bioreactors were operated to establish low to high convective flows towards the ECS. At the end of the culture cells were processed for Scanning Electron Microscopy (SEM). Histological sections were stained with H&E to evidence cell radial organization and with DAPI to analyze cell nuclei distribution along the bioreactor length. Osteoblast phenotype maintenance was confirmed by pro-collagen immuno-staining, collagen deposition detected with Masson–Goldner stain.
Results: HFMB operated under low convective flows presented a uniform axial cell distribution and poor cell proliferation. Cells were found at the membrane surface forming a thin layer around them. SEM analysis confirmed the low proliferation rate under this operating condition. Histological analysis showed how in the presence of high spontaneous convective flows cells were dragged at the bioreactor outlet. Despite this evidence, this operating condition seems to be the most promising in obtaining large bone tissue engineered substitutes as demonstrated by the rearrangement of ostegenic cells in aggregates 2.5cm thick. SEM analysis showed how cell number grew significantly respect to the seeding density forming a continuous thick multilayer covering the surface of several adjacent membranes.

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Conclusions: A delocalized and distributed nutrients supply coupled to bioreactor design promoting the occurrence of high Starling flows in the cell compartment may contribute to obtain clinical-scale 3D tissue engineered constructs.

P167 (EI0147)
POTENTIAL OF MESENCHYMAL STROMAL CELLS DERIVED FROM HUMAN ADULT ADIPOSE TISSUE AND BONE MARROW FOR REGENERATIVE MEDICINE AND TISSUE ENGINEERING
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Objectives: The aim of this study was to assess the clonogenic efficiency, immunophenotype and the potential of adipose tissue (AT) and bone marrow (BM) mesenchymal stromal cells (MSC) for *in vitro* differentiation into different mesenchymal and non-mesenchymal lineages.
Methods: Human AT and BM were obtained with the patient informed consent under full Ethical guidelines. Immunophenotype of cells was determined by flow cytometry. The efficiency of adipogenic and osteogenic differentiation of cells was determined *in vitro* in media, supplemented with specific induction factors. Endothelial potential of MSC at different stages of culture was assessed using Matrigel assay. Differentiation of MSC into insulin-producing cells was prepared during culture in high glucose medium in the presence of pancreatic stimuli.
Results: It was found that clonogenic efficiency of AT and BM derived MSC at passage 1 comprised 8.1±1.8% and 8.5±2.3%, correspondingly. The analysis of the adipogenic differentiation efficiency showed no significant differences in the percentage of differentiated cells as well as in triglycerides content between two MSC sources. The accumulation of extracellular calcium was 1.23 times higher in BM MSC cultures compared to AT MSC after osteogenic differentiation. Additional clonal analysis of AT MSC showed that about 50% of clones have the ability for osteogenic differentiation. MSC at different stages of cultivation were able to form capillary-like structures in the Matrigel confirming the endothelial differentiation of cells. Differentiation of AT MSC and BM MSC into insulin-producing cells resulted in the formation of cluster-like aggregates and insulin expression, confirmed by PCR, ELISA and immunocytochemistry. After seeding into macroporous alginate-gelatin scaffolds, MSC from both sources demonstrated capability to proliferation and multilineage differentiation.
Conclusions: MSC, derived from both BM and AT are promising for regenerative medicine and tissue engineering applications; however, each of them has advantages and drawbacks for a special purpose that will be additionally discussed.

P168 (EI0204)
EFFECT OF COMPRESSIVE PLANE STRAIN ON OSTEOBLAST-LIKE CELLS *IN VITRO*
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Objectives: The aim of this research was to verify that a novel bioreactor was able to exert compressive stimulation on cells. Osteoblast-like cells were chosen as their normal environment is compressive and the cells themselves are not contractile. The bioreactor developed in this research is intended to give a useful and repeatable means to investigate the effect of compressive strain on the mechanobiology of osteoblasts.
Methods: A novel bioreactor was used to apply cyclic compressive mechanical strain to cultured MG63 human osteosarcoma cells. Cells were seeded onto flexible polyurethane membranes and maintained in static culture in DMEM for 48 hours. The cells were then cultured for a further 48 hours while being stimulated by intermittent cyclic strain (1 hour at 30 cycles per minute), with strain magnitudes of up to 2%, then allowed 24 hours before fixation for imaging or protein analysis. At the conclusion of the experiment, cells were imaged using phase contrast microscopy and scanning electron microscopy, and protein expression was examined by PCR.
Results: The results confirm the prediction by the classical theory that the concave face of a cantilever beam will be in compression. The effects of cyclic tensile and compressive strain on cell morphology, cell metabolism and protein expression are presented. Higher strain magnitudes were found to result in apoptosis, in accordance with the literature.
Conclusions: The bioreactor developed in this research is able to exert compressive stimulation at physiological magnitudes on the cells studied. Stimulation of cells in conditions similar to those encountered *in vivo* will enhance our understanding of the mechanobiology of mammalian cells.

P169 (EI0081)
PRECONDITIONING OF ADIPOSE TISSUE-DERIVED MESENCHYMAL STEM CELLS WITH NATURAL MOLECULES FOR VASCULAR CELL THERAPY
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Objectives: Peripheral arterial disease represents a major health problem in modern society. If peripheral vascular occlusion progresses to ulceration or gangrene, the risk of limb loss becomes substantial. In patients where no medical treatment is considered effective for rest pain or ulcer healing, cell-based therapeutic angiogenesis has become a new promising hope. It has been reported that adipose tissue contains progenitors with angiogenic potential and that therapy based on Adipose-Derived Mesenchymal Stem Cells (ADMSCs) administration can improve perfusion recovery in hindlimb ischemia mouse model. Here we aimed to enhance and optimize both vascular and perivascular commitment and paracrine patterning of human ADMSCs using natural molecules *in vitro*.
Methods: ADMSCs were isolated from lipoaspirates, characterized, and treated with hyaluronic (HA), butyric (BU), and retinoic (RA) acid. Vasculogenic genes expression, including VEGF, KDR, HGF, and HIF-1α, was analyzed by Real-time PCR at 1-2-3-6 days. The presence of vascular (vWF) and perivascular (NG2, a-SMA, CD146, PDGF-Rβ) markers was evaluated by immunofluorescence and flow cytometry at 14 days. Secretion of angiogenic cytokines (VEGF, HGF) was assessed by ELISA.
Results: Combinatorial treatment with HA, BU, and RA significantly increased the transcription of vasculogenic genes at every time point, and induced vWF expression, that is not detectable in untreated cells. The treatment remarkably augmented the percentage of cells expressing perivascular markers, and enhanced the secretion of angiogenic factors compared to control cells. Expression of PDGF-Rb, involved in proliferation of undifferentiated cells, is dramatically reduced in HA-BU-RA treated cells, suggesting a cellular commitment.
Conclusions: ADMSCs represent an attractive alternative source of pluripotent cells compared to bone marrow in terms of accessibility and available tissue amount. The availability of natural molecules to enhance the endothelial and perivascular commitment of these cells may constitute a promising therapeutic approach for cell therapy in patients with ischemic vascular diseases.

P170 (EI0097)
REGENERATION OF THE INTERVERTEBRAL DISC (IVD) USING *IN VITRO* DIFFERENTIATED STEM CELLS
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Objectives: The degeneration of the nucleus pulposus (NP) is one major reason for low back pain. One possible method of treatment is cell-based therapy with differentiated human mesenchymal stem cells (hMSC). For a differentiation into NP cells, the hMSCs require a 3D environment and various stimuli such as growth factors. In our work, we want to identify the optimal stimulation for the differentiation of hMSCs into NP cells. Regarding the therapeutical use it is indispensable to verify the differentiation success of hMSCs into NP cells and to delimit the differentiated cells to chondrocytes. As Raman spectroscopy has a high potential for non-invasive characterization of suspension cells and distinction between different cell lines, our research group together with the Fraunhofer Institute of Interfacial Engineering and Biotechnology (IGB) tested the applicability of this method for cells embedded in hydrogels.
Methods: hMSCs were cultivated three-dimensionally to form NP cells. To investigate the putative differentiation stimulating ability of several growth factors and components of the extracellular matrix (ECM), RT-PCR as well as fluorescence immunostaining of NP-specific marker proteins were done.
Results: In all differentiation experiments with growth factors, NP-specific marker proteins were expressed. Data concerning the differentiation of hMSC under the influence of ECM components will be presented. Using Raman Spectroscopy as well as common methods of molecular biology, we were able to distinguish NP cells and differentiated hMSC from undifferentiated stem cells and chondrocytes.

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Conclusions: The expression of NP-specific marker proteins indicates the ability of three growth factors to differentiate hMSCs into NP-like cells. Using Raman spectroscopy and RT-PCR we could clearly display that NP cells differ from chondrocytes. As only pure NP cells (e.g. into NP cells differentiated hMSCs) could be used for subsequent therapeutical use, this finding is of great benefit for NP regeneration approaches.

P171 (EI0077)

MODULATION OF ANGIOGENESIS BY FIBROBLASTS IN TISSUE REGENERATION

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Objectives: Promoting angiogenesis in a damaged tissue is a major challenge for tissue regeneration. It is important to observe and understand the morphology and the ability of cells to adapt to new biomaterial systems. The immobilization of cocultured bone progenitor cells with ECs within RGD-alginate has shown promising results as a bone regeneration strategy. In the present work, it is hypothesized that fibroblasts influence ECs and can thus improve angiogenesis.
Methods: *In vitro* studies were carried out by investigating the influence of fibroblasts immobilized in a RGD-alginate matrix on EC assembly into capillary-like structures. An *in vivo* Matrigel plug assay was used and Hemoglobin levels and inflammatory factors were determined.
Results and Discussion: *In vitro* studies showed that the presence of fibroblasts supported capillary-like structures formation for longer periods than controls (without fibroblasts). The length of the capillary-like structures were longer in the presence of immobilized fibroblasts compared to control conditions. *In vivo* studies using Matrigel plugs demonstrated that the presence of fibroblasts increased hemoglobin levels, although no significant differences were observed concerning N-acetylglucosaminidase activity and nitric oxide production, compared to controls (without fibroblasts).
Conclusions: The present findings indicate that fibroblasts improved angiogenesis and did not seem to influence the inflammatory process in serum. Fibroblasts immobilized in RGD-alginate maintained their capacity to enhance angiogenesis. These findings provide evidence for the potential use of this strategy in tissue regeneration where vascularization is essential.

P172 (EI0090)

ORAL SKELETAL TISSUE AS AN APPLICABLE SOURCE OF PROGENITOR CELLS FOR REGENERATIVE MEDICINE

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Objectives: Tissue engineering is a promising approach for regenerative procedures in oral and maxillofacial surgery. This study investigated the suitability of oral skeletal tissue as an applicable source of progenitor cells and an alternative to the iliac crest bone marrow. The aim was to compare multilineage differentiation potential of osteoprogenitor cells and bone marrow mesenchymal stem cells (BM-MSCs).
Methods: Osteoprogenitor cells were isolated from explant cultures of intraorally harvested bone chips during routine oral surgery. BM-MSCs were obtained from iliac crest bone marrow aspirates and used as positive control for multilineage differentiation analysis. Cells were immunocytochemically characterized by the expression of characteristic surface antigens including CD73, CD90, CD105 and the lack of CD14, CD34, CD45. Differentiation capacities into the osteogenic, adipogenic and chondrogenic lineages were investigated using cytochemical tests (alkaline phosphatase activity, Oil Red O and Alcian blue staining) and RT-PCR analysis.
Results: Osteoprogenitor cells showed characteristics of BM-MSCs like plastic adherence and expression of defined surface antigens. Their differentiation capacity into the osteogenic, adipogenic and chondrogenic lineages was comparable to the one of BM-MSCs.
Conclusions: These findings suggest that osteoprogenitor cells have a similar differentiation potential to BM-MSCs *in vitro*. Oral skeletal tissue may be considered as a suitable source of cells for tissue engineering therapies in regenerative dentistry.

P173 (EI0067)

THE REVERSE REMODELLING EFFECT OF BONE MARROW-DERIVED STEM CELLS IS INDEPENDENT FROM THE SITE OF EPIMYOCARDIAL CELL TRANSPLANTATION

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Objectives: The transplantation of bone-marrow derived stem cells represents a promising therapy in chronic heart failure. Positive effects of transplanting these cells could be shown, but the exact mechanisms are unknown. As paracrine effects are increasingly discussed, we evaluated if the injection site effects the amelioration on LV-contractility and angiogenesis in doxorubicin-induced failing hearts.
Methods: Heart failure was induced in White New Zealand rabbits by doxorubicin (3mg/kg for 6 weeks), followed by right-ventricular-transplantation (RV-BMC, n=6), left-ventricular-transplantation (LV-BMC, n=6), sham treatment (medium-group, n=6), or no therapy (DOX, n=5). Healthy rabbits were used as controls (control-group, n=8). Cells were isolated by bone marrow aspiration and transplanted locally in the ventricle. Four weeks later the cardiac function was assessed, and capillary density (CD31-staining) was measured.
Results: The ejection fraction was significantly higher in both BMC-groups vs. medium-group (LV-BMC 39.0±1.4% vs. medium-group 29.8±3.7% p<0.001, RV-BMC 39.2±2.6% vs. medium-group 29.8±3.7% p=<0.001), without significance between the BMC-groups (p=0.858). The capillary density (capillaries/high-power-field) increased in both BMC-groups in all chambers of the heart compared to medium group. The left ventricular injection of BMCs increased the capillary density by 8.3±3.4%, the right ventricular injection by 8.1±2.2% compared to medium group without significant difference between the two BMC-groups.
Conclusions: The beneficial effects of BMC transplantation in doxorubicin-induced cardiomyopathy are independent of the injection site. As BMCs failed to transdifferentiate into cardiomyocytes, paracrine factors seem to be responsible for the beneficial effects of stem cell transplantation.

P174 (EI0070)

CREATININE TRANSPERITONEAL TRANSPORT *IN VITRO* - INFLUENCE OF P-CRESOL AND METHYLGLYOXAL

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Objectives: Functional and morphological modifications of peritoneum have been observed during long-term dialysis, peritonitis and cancer. Creatinine transfer is used as a marker to estimate changes in peritoneal membrane. P-cresol can be considered to be compounded with several toxic and some beneficial properties connected e.g. with protective antioxidative effects. Similarly, methylglyoxal is a well-known reactive carbonyl solute with probably antiviral and anticancer properties.
Methods: The object of study were analyses of p-cresol and methylglyoxal influence on creatinine transport across peritoneum *in vitro*. Membrane isolated from anterior abdominal wall of white New–Zealand rabbits, modified Ussing-type chamber and mathematical model of mass transfer were used to calculate the diffusive permeability coefficient P[cm/s] in the case of transport directed from the interstitial (I) to the mesothelial (M) side of the membrane and in the opposite direction. Four separate series of the experiments were done. In the first and second one (control conditions) we examined the rates of the creatinine transport in the concentration gradients: 0.1g/dL and 0.01g/dL, respectively, during 120 minutes. In the next, values of P for creatinine (0.1g/dL) before (15-60minutes) and after p-cresol (0.005g/dL) introduction into the experimental system (75-120min) were investigated. In the fourth series, transfer of creatinine (0.01g/dL) before (15-60min) and after methylglyoxal (0.01g/dL) applications (75-120minutes) was analyzed.
Results: Dynamics of the creatinine transport in both concentration gradients remained constant. The values of P+/-standard error of the mean were 2.340+/-0.265[x0.0001;cm/s] and 2.381+/-0.244[x 0.0001;cm/s] for I->M and M->I, respectively. Introduction of p-cresol into the experimental system did not alter the transfer of creatinine, both in the case of I->M and M->I passage. In contrast, methylglyoxal caused a 7% (p<0.01) decline of the bidirectional transport of examined solute.
Conclusions: *In vitro* methylglyoxal, but not p-cresol, modifies the diffusive permeability of peritoneum for creatinine. Observed results may be clinically important.

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P175 (EI0029)

IN SILICO STUDY OF AN INNOVATIVE MICROGRAVITY PERFUSION BIOREACTOR FOR HYDROGEL-BASED CARDIAC REGENERATIVE MEDICINE

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Objectives: In cardiac regenerative medicine, hydrogel-based injectable scaffolds (hydrogel) are becoming a promising strategy for supporting the regeneration of injured heart. The rationale for this study is to assist the design of an innovative low-cost perfusion bioreactor for cell-seeded hydrogel feasibility testing, in which microgravity condition is realized by establishing a mixing slow vortex that allows adequate cell-seeded hydrogel suspension and oxygen transport without using rotating components. Computational fluid dynamics was applied to assist the bioreactor design and to identify the operating conditions that optimize mass transport in the culture chamber.
Methods: The finite volume method was applied to simulate 3D multiphase (culture medium, cells, oxygen) fluid-dynamics, integrating calculations of diffusion, convection and consumption for assessing *1)* the optimal geometric design, *2)* the proper flow regime to be established within the culture chamber, and *3)* the oxygen distribution and its consumption by cells.
Results: Remarkable differences in the cell-seeded hydrogel distribution and suspension, in the shear stress distributions, and in the oxygen distribution and consumption arise due to variations in perfusion parameters. Our main findings are the optimization of the geometry of the chamber and the identification of a range of flow rate values that *1)* allow cell-seeded hydrogel suspensions, avoiding sedimentation at the bottom of the chamber, *2)* guarantee a safe range of shear stress values on cells, and *3)* permit appropriate oxygenation.
Conclusions: The present study allowed to properly design an innovative low-cost perfusion bioreactor (without rotating components) for cell-seeded hydrogel culture in microgravity conditions, ensuring homogenous distribution of cell-seeded hydrogel and adequate oxygen cellular consumption, and avoiding shear-induced cell damage. Findings from computational simulations will serve as criteria to set the operating conditions for future *in vitro* tests. The present work is carried out in the scope of BIOSCENT Project (ID 214539).

P176 (EI0018)

HEPATIC CELL ENCAPSULATION AS A 3D CULTURE MODEL FOR THE STUDY OF HEPATITIS C VIRUS

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Objectives: Since the discovery of hepatitis C virus (HCV), the lack of relevant cell culture system has hampered research progress on this widespread human pathogen. New approaches in tissue engineering could be a physiologically relevant hepatocyte culture model and enable a broad range of basic and applied studies to be achieved. To prolong and strengthen this breakthrough, the use of the fluidized bed bioreactor for hepatic cell cultured in alginate beads is investigated to, on the one hand mimic *in vivo* cellular conditions by the 3D environment and on the other hand, promote HCV permissiveness and viral production.
Methods: Hepatic cells were encapsulated in four compositions (low viscosity, medium viscosity, at 1 or 2%) of alginate beads (500µm). The growth kinetics of a human hepatocyte line, Huh-7.5.1 cells, in alginate beads were followed in dynamic condition by DNA quantification. Albumin concentration was determined by an ELISA assay to study the cell functionality. The important factors for HCV infection (HCV receptors, tight-junction, and polarity markers) were studied using immunofluorescence analysis and confocal microscopy. 3D culture of Huh-7.5.1 was infected with JFH-1 HCV and HCV-RNA levels in culture supernatants were quantified by Q-PCR.
Results: The alginate encapsulation increases Huh-7.5.1 cell density, supports proliferation, liver function as compared to the 2D cell culture system. We demonstrate that depending of the composition of alginate, Huh-7.5.1 cells cultivated in beads formed 3D-complex, particularly aggregates and multicellular channel-like structures. This cellular organization may influence expression and relocalization of tight junction, polarity markers, HCV receptors, in comparison with 2D culture. Importantly, the multicellular structures may remain highly permissive for HCV infection.
Conclusions: These data are encouraging to open the HCV potential accessibility to hepatocytes *in vitro*. Encapsulated Huh-7.5.1 cells may represent a promising physiologically relevant system for further *in vitro* studies of HCV life cycle, host–virus interactions.

P177 (EI0302)

BIODEGRADABLE THERMOPLASTIC POLYURETHANES: SYNTHESIS AND CHARACTERIZATION OF IMPROVED TPUS

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Objectives: Thermoplastic polyurethanes (TPUs) are mechanically elastomeric, have good biocompatibility, and are amenable to be processed from melt or solution. These characteristics make TPUs particularly interesting in soft tissue engineering. While biodegradation of surgical implants has been traditionally viewed as a negative characteristic, it can be a potentially very useful property if the rate of degradation is well controlled to correlate with the regrowth of native tissue. To better understand and hopefully exploit this synergetic phenomenon, our group wishes to produce biodegradable thermoplastic polyurethanes with improved mechanical properties and study the biodegradation behavior.
Methods: To increase the tensile stength of the existing TPUs, the aliphatic diisocyanate has been replaced with a more rigid alicyclic diisocyanate. Alicyclic TPUs are expected to have comparable mechanical performance to commercially available aromatic TPUs with reduced toxicity of amine degradation products. We also tried to impact the decomposition time by varying the length of the softblock and by using a degradable polyester (caprolactone) instead of polyTHF.
Results: The new produced TPUs are organic soluble allowing molecular weight (from 30k to 70k M_n) analysis by GPC. Bulk mechanical properties (modulus, tensile strength, and percent elongation) are tested in tensile tests. Simulated biodegradation is tested *in vitro* with and without enzymes. A terephthalic based chain extender has already been shown to undergo slow degradation at elevated pH with prolonged degradation times of one to two years.
Conclusions: There are many possible application areas for these new synthesized biodegradable TPUs like all areas of soft tissue engineering, drug delivery systems, disposable medical tubing, packaging and textiles.

P178 (EI0261)

PULSATILE FLUID FLOW ENHANCES ENGINEERED BONE DEVELOPMENT BY HUMAN ADIPOSE DERIVED STEM CELLS

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Objectives: Within native bone, bone cells experience mechanical shear force due to the flow of interstitial fluid generated by physical movement. Bone tissue engineering could greatly benefit from mimicking such physiologic environment, in which the interstitial flow is dynamic and associated with fluctuating shear stress. To this end, we investigated the effects of pulsatile fluid flow (PFF) regime on bone formation *in vitro* by human adipose stem cells (hASCs) on porous three-dimensional scaffolds, in contrast to conventional continuous fluid flow (CFF). We also determined the timing of application of PFF that results in most rapid osteogenesis of hASCs.
Methods: Porous silk-fibroin scaffolds (400-600µm pore size) were seeded with hASCs ($30x10^6$cells/mL) and cultured in osteogenic medium under four distinct fluid flow regimes: 1) PFF, for 5 weeks; 2) CFF, 1 week, PFF, 4 weeks; 3) CFF, 2 weeks, PFF, 3 weeks; 4) CFF, for 5 weeks. PFF regime was applied at flow velocities ranging from 400µm/s-1200µm/s, at 0.5Hz frequency, for 2h every 10h, with the CFF regime during the remaining culture times. In all groups, CFF was applied at 400µm/s.
Results: Constructs cultured in the CFF regime demonstrated inferior bone development in terms of cell proliferation, bone protein deposition and calcification, as compared to constructs subjected to PFF. The best tissue development was achieved in group 3, when hASC were pre-differentiated for 2 weeks under CFF, and then subjected to the PFF regime, as evidenced by maximum values of bone protein deposition, calcium content, bone volume, and expression of PGE2 mechanotransduction marker.
Conclusions: Over 5 weeks of culture, PFF improved bone formation over CFF. The response of hASCs to PFF was enhanced following pre-differentiated into the osteogenic lineage. We thus propose that hASCs develop a mechanism to detect and respond to change in shear force by progression of osteogenic differentiation.

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P179 (EI0150)

A NUMERICAL MODEL OF MASS AND MOMENTUM TRANSPORT IN CONVECTION-ENHANCED HFMBS FOR LONG BONE TISSUE ENGINEERING

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Objectives: The synergistic effects of convection and a distributed and delocalized nutrient transport in hollow fibre membrane bioreactors(HFMBs) have been recently reported to benefit the culture of cm-scale BMSC aggregates, possibly by relieving nutrients limitations typical of other bioreactors for bone tissue engineering (BTE). Mathematical modelling of mass transport, cells growth and metabolic reactions is particularly interesting given the difficulty to monitor non-invasively solutes concentration in the presence of a closed shell, to optimize bioreactor design and operation. Most proposed models consider cells uniformly distributed in the extracapillary space, in contrast with experimental results under high convective flows, and are then inadequate for this purpose. This paper presents mathematical models of nutrients profiles inside HFMBs operated in close shell mode from diffusion-limited to convection-dominant mass transport conditions for both uniform cell distribution and the actual non-uniform cell distribution observed in experiments with BMSCs.
Methods: Models are based on a multi-compartment description of HFMBs based on the Krogh cylinder assumption, and on a quasi-steady state analysis of nutrients evolution and cell concentration profiles. Relevant non-dimensional parameters were identified, and governing momentum and mass transport equations were numerically solved with a finite element commercial code. Metabolic parameters for primary and immortalized cell were used, proliferation and distribution parameters were assessed from culture experiments.
Results: The use of low metabolic requirement cell types, like immortalized ones makes the design of bioreactors for 3D constructs culture easier, being diffusion-limited nutrients transport models inadequate only for cell density close to natural bone tissue. Simulation results demonstrate the importance of convective nutrient transport, membrane permeability and packing density in the cell compartment on nutrients concentration in the ECS when primary cells are used.
Conclusions: Convection-dominant nutrients transport is necessary to overcome nutrient transport limitation when culturing cells types with physiological metabolic requirements in 3D cm-scale constructs.

P180 (EI0211)

PRELIMINARY STUDY ON DEVELOPMENT OF THE SERIES OF THE HOLLOW FIBER MEMBRANE BIOREACTORS (DEVOTED TO DIFFERENT CELL CULTURE APPLICATIONS)

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Objectives: Hollow fiber bioreactors have broad spectrum of applications including biotoxicity testing and tissue engineering studies. Structure of the membrane should enable exchange of the biochemical compounds with required molecular mass between the inner and outer bioreactor compartments. In our institute an innovative method of modification of the standard polysulfone membranes was elaborated aiming at a controllable increase of the membranes cut off (MWCO). The objective of this preliminary study was to evaluate filtration properties of series of the prototype, modified, polysulfone membranes designed for the application in the cell bioreactor.
Methods: Four groups of membranes: A, B, C (subgroups: C1, C2, C3) and D placed inside the bioreactor casings were tested under *in vitro* conditions. Modification of the membrane structure was performed based on change of the developed original technological procedure. Membranes were evaluated with saline using ultrafiltration studies (quantitative assessment) and in the separation circuit with human plasma (qualitative assessment). Sieving coefficients for glucose, albumin, IgG, HDL, IGM, and LDL were calculated and then MWCO was estimated.
Results: The results for the ultrafiltration coefficient were as follows: 6, 28, 13, 18, 30 and 56 mL/(min*mmHg*m^2), for membrane type A, B, C1, C2, C3 and D, respectively. The lowest value of MWCO = 80kDa was obtained for bioreactor with type A membrane. In the remaining groups, the following MWCO values were obtained: B – 350kDa, C1 – 110kDa, C2 – 150kDa, C3 – 2000kDa and for type D membrane MWCO was greater than 2700kDa.
Conclusions: The results demonstrated that MWCO increased as a response to the modification of the membrane structure (MWCO ≥ 80 kDa) in comparison to standard, not modified polysulfone membrane characterized by the MWCO < 69 kDa. It seems that the developed method makes it possible to prepare the membranes with required permeability appropriate for specific bioreactor applications.

P181 (EI0411)

PLATELET LYSATES AS A SCAFFOLD COMPLEMENT PROMOTING HASCS PROLIFERATION AND OSTEOGENIC DIFFERENTIATION

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Objectives: This work aims to establish Platelet Lysates (PL) as optimal source of growth factors and other molecules that are vital for promoting cell proliferation and differentiation pathways, eventually allowing the substitution FBS and/or osteogenic supplements in culture media in bone tissue engineering strategies. Furthermore we intend to design new approaches to incorporate PLs in a scaffold material, as a hydrogel encapsulating the cells or as a coating for 3D porous structures, thus developing a tissue engineered construct with enhanced/multiple functionalities.
Methods: Starch–polycaprolactone (SPCL) meshes were obtained by a fiber bonding method as previously described. PL gels were obtained by activation of platelets coagulation cascade using thrombin dissolved in a calcium chloride solution. Human adipose stem cells (hASCs) were obtained by enzymatic digestion of lipoaspirates samples. hASCs were either seeded directly into the SPCL scaffolds (control group) or into the scaffolds previously coated with PL gels or suspended in the PL and then seeded in the scaffold and gellified. hASCs proliferation and differentiation was assessed after different culturing time points of the constructs, by DNA and ALP quantification and by RT-PCR and immunohystological analysis.
Results: The preliminary results obtained sustain the hypothesis that growth factors and other signaling molecules present in PL groups are actually active and vital to initiate proliferation and osteogenic differentiation of hASCs. DNA quantification and cell viability were similar and even higher in PL groups, as well as early markers of osteogenic differentiation, such as ALP activity. Latest time-points revealed less noteworthy differences especially due to the progressive degradation of the PL gel.
Conclusions: PL represents a substrate and a delivery system of important growth factors and other signaling molecules, and therefore making these molecules available for cells within a tissue engineering construct provides an important enhancement of autologous bone tissue engineering strategies.

P182 (EI0370)

BUILDING THE BASIS FOR HUMAN MENISCUS REGENERATION

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Objectives: Total or partial meniscectomy has been the gold standard for the treatment of degenerated/diseased menisci. Despite meniscal regeneration represents a recent trend in tissue engineering, fundamental studies related to human meniscus biochemistry and biomechanics are still scarce. This work aims to contribute in the knowledge of this tissue aiming at future clinical applications, namely the aspects dealing with the cellular phenotypes and density, biomechanics and extracellular matrix composition.
Methods: Human tissue was obtained from local hospitals by means of surgery or biopsy, in accordance with local ethical committee guidelines. The HMC's were isolated from different donor (sex and age) explants or using an enzymatic standard protocol. Micro-computed tomography (Micro-CT) of freeze-dried meniscus was carried out. Histological (haematoxylin and eosin - H&E, trichrome stain and toluidine blue stainings) analysis was performed for segmental characterization of ECM and cells density. Dynamic mechanical analysis was carried out for medial, anterior and posterior segments of meniscus (in PBS at pH 7.4).
Results and Discussion: Micro-CT analysis revealed that meniscus (freeze-dried) possessed a mean porosity of 53%, a mean pore size and trabeculae thickness of 85μm and 80μm, respectively. The cells isolated from meniscus are

a mixed population of cells, *i.e.* fibrochondrocyte-like and MSCs. The histological evaluation has shown that meniscus ECM is composed of collagen-type I. This tissue is fibrocartilagineous in nature and presented a higher cell density in the periphery as compared to meniscus core. Cellular density among the different segments (anterior, medial, posterior) of meniscus was quantified using the H&E 2-D histological images.
Conclusions: This study has contributed to improve the knowledge on meniscus biology and mechanical properties. It is believed that these important issues should be considered to develop adequate acellular and cellular strategies for tissue engineer meniscus.

P183 (EI0079)
COMBINING OPTICS AND ULTRASOUND TO IMAGE 3D TISSUE CONSTRUCTS
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Objectives: Tissue scaffolds are an integral part of the tissue engineering process, assisting in the culturing of cells in three dimensions. It is important to understand both the properties of the scaffold and the growth of cells within the scaffold. This paper describes a system to characterise scaffolds using acoustic techniques alone and the development of an ultrasound-modulated optical tomography system to study the growth of cells within the scaffolds. The ultrasound modulated system allows the effects of light scattering in relatively thick tissue constructs (several mm) to be reduced.
Methods: Acoustic techniques alone have been applied to characterise foamed scaffolds manufactured from synthetic polyesters poly(lactic acid) (PLA) and poly(lactic-co-glycolic acid) (PLGA) via a supercritical fluid process. An ultrasound modulated optical tomography system has been used to image absorbing and fluorescent objects in gel scaffolds.
Results: Although foamed scaffolds are porous and therefore highly scattering to sound waves, results demonstrate that acoustic signals are detectable through a 6mm thick foamed scaffold. Images of optically-absorbing materials embedded in gel-based tissue phantoms will be presented demonstrating that a lateral resolution of 250μm and an axial resolution of ~90μm can be achieved in scattering samples. Preliminary results of non-linear acousto-optic modulation will also be presented.
Conclusions: Combining optics and ultrasound can be used to obtain high-resolution optical images of highly scattering, thick tissue constructs.

P184 (EI0086)
REGULATION AND CHARACTERISATION OF CORNEAL STROMAL CELL CONTRACTION
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Objectives: Collagen hydrogels have been extensively used as scaffolds for corneal tissue engineering. However, corneal stromal cells differentiate into contractile fibroblasts in the hydrogel *in vitro* culture, rather than keratocytes. The aim of this study is to develop techniques to regulate the contraction by either chemical or topographical cues which mimic the native corneal environment, and characterize the cellular feedback in prolonged culture periods via novel, non-destructive monitoring protocols.
Methods: $5x10^5$ human corneal stromal cells were seeded in collagen hydrogels with and without the incorporation of poly-lactic acid aligned nanofibers. A non-destructive spherical indentation technique was used to examine the alteration of the mechanical properties of the individual collagen hydrogel specimens under different media respectively up to 28 days. The dimensional change of the specimens caused by the cells' contraction was measured by optical coherence tomography in parallel. The quantitative-PCR with respect to the expression of keratocytic and fibroblastic markers was conducted to cross-validate the observed physical properties. It was revealed that stromal cells cultured under media with insulin and without serum exhibited constant elastic modulus and gel dimension, indicating that contraction was suppressed, which was cross-validated by the expression of keratocan and ALDH3; whilst stromal cells cultured with serum demonstrated continuously increased modulus and reduction of thickness, typical of contraction process. The presence of aligned nanofibers reduced the degree to which the cells were able to contract the hydrogel constructs in a vertical direction, thus encouraging the cells cultured in fibroblastic media to behave more like non-contractile keratocytes.
Conclusions: The alteration of culture conditions and the addition of topographical cues can regulate corneal stromal cell differentiation. This can potentially enhance the field of corneal tissue engineering using collagen hydrogel models. The non-destructive monitoring protocols provide convenient tools for observing biological phenomenon for prolonged culture periods in the same specimen.

Tissue Engineering of Skin

P185 (EI0366)
ATTACHMENT AND SPREADING OF FIBROBLASTS ON SELF-ASSEMBLING BIOACTIVE MATRICES
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Objectives: The primary objective of this work was to investigate the potential of 2D biodegradable membranes as supportive bioactive matrix for wound healing by studying the behavior of human fibroblasts on these membranes. Towards this goal, we developed a biomimetic matrix that incorporates structural components of skin extracellular matrix (hyaluronan) and biochemical signals (RGDS epitope) to recreate some aspects of skin tissue niche. The RGDS sequence is present in cell binding domains of extracellular proteins (such as fibronectin) and is known to promote integrin-mediated cell adhesion.
Methods: The proposed bioactive matrices result from the self-assembly between peptide amphiphiles and hyaluronic acid (HA), a major component of skin ECM. The RGDS sequence was incorporated in the peptide structure to provide the matrices with cell-adhesive properties. Cell culture was then performed and the effect of the RGDS epitope on the adhesion, morphology and proliferation of primary human dermal fibroblasts was followed respectively by, scanning electron microscopy, immunostaining and DNA quantification.
Results: Cell responses to RGDS matrices were compared to matrices containing DGSR (scrambled sequence that does not promote cell adhesion). When cultured on membranes without the cell recognition epitope RGDS, human dermal fibroblasts showed lower adhesion to the matrices when compared to the ones containing RGDS. SEM analysis showed adherent cells on the RGDS matrices and the presence of filopodia which are known to be involved in the regulation of cell migration.
Conclusions: We expect that the proposed biodegradable bioactive matrices could offer significant potential in skin regeneration strategies and also as model systems for fundamental mechanistic studies in wound remodeling.
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P186 (EI0307)
AN ARGININE INCORPORATED NANOCOMPOSITE POLYMER FOR CARDIOVASCULAR IMPLANTS
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Objectives: Cardiovascular implants must resist thrombosis. Polyhedral- oligomeric-silsesquioxane-poly(carbonate-urea)urethane (POSS-PCU) nanocomposite polymer has demonstrated suitable properties for cardiovascular applications. L-arginine is recognised as a significant amino acid with anticoagulant properties with a link to nitric oxide synthesis. Water soluble arginine is immobilized within the polymer via nanoparticles thus presenting a novel surface modification method for blood contacting polymers. The study aims to determine the antithrombogenic properties of Arginine-POSS-PCU.
Methods: Arginine was reacted with amine functionalized fumed silica nanoparticles using fmoc chemistry and incorporated into POSS-PCU at 5-8%W/W. Surface properties of Arginine-POSS-PCU samples were determined using FTIR and XPS. A thorough investigation of whole blood kinetics on Arginine-POSS-PCU was performed with Thromboelastography polymer coated cups. Platelets were introduced onto Arginine-POSS-PCU samples and incubated for 90mins at 37°C on a shaker before platelet adhesion morphology with SEM and the changes in platelet activated factors were determined with ELISA. Plasma from whole blood was also introduced onto Arginine-POSS-PCU and the changes in protein ad-

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sorption were also determined using fibrinopeptideA and PreKallikein assay.
Results: Surface presence of arginine on Argnine-POSS-PCU was confirmed. Thromboelastography tests relvealed that arginine-POSS-PCU had a lower rate of initial clot formation, (determined with K/min,R/min, α/deg) as well as longer time and a slower rate for maximum thrombus generation (TMRLG/min,MRTG/mm/min) SEM demonstrated less platelet adhesion with a more round morphology with least pseudopodia.PF4 ELISA demonstrated least activation of platelets. Fibrinopeptide A and PreKallikrein assays demonstrated that thrombogenic plasma proteins are influenced by arginine-POSS-PCU with lowered proteins.
Conclusions: Arginine-POSS-PCU cardiovascular grafts instill greater antithrombogenic properties.

P187 (EI0305)
LIPOSOME-ENCAPSULATED HEMOGLOBIN ACCELERATES SKIN WOUND HEALING IN MICE
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Objectives: Effects of liposome-encapsulated hemoglobin with moderately high O_2 affinity (m-LEH, $P_{50}O_2$=17 mmHg) on the skin wound healing were examined in mice.
Methods: Two full-thickness dorsal wounds, 6mm in diameter, with a surrounding silicone stent were created in C57BL/6J mice. Two days later (Day 2), animals randomly received intravenous m-LEH (2mL/kg, n=12), homologous blood transfusion (RBC, n=11) or saline (SL, n=12). The same treatment was repeated 4 days after wounding, while the sizes of the skin defect and ulcer were monitored on Days 0, 2, 4 and 7, when all animals were euthanized for morphological studies.
Results: While the size of the skin defect in relation to the stent ring remained the same in all the groups, the size of the ulcer compared to the skin defect or silicone stent became significantly reduced on Days 4 and 7 in mice treated with m-LEH (46±10% of pretreatment size, P<0.01) compared to mice treated with RBC transfusion (73±6%) or saline (76±7%). M-LEH treatment significantly accelerated granulation, increased epithelial thickness, suppressed early granulocyte infiltration, and increased Ki67 expression in accordance with the ulcer size reduction, while there was no difference in surface blood flow as determined by Laser-Doppler flow meter or CD31 expression by immunohistochemical staining among the groups.
Conclusions: The results suggest that m-LEH (2mL/kg) may accelerate skin wound healing in C57BL/6J mice via mechanism(s) involving reduced inflammation and increased metabolism, but not by improved hemodynamics or endothelial regeneration.

P188 (EI0281)
DEVELOPMENT OF DERMAL BILAYERED SCAFFOLD WITH NEW GENERATION OF NANOCOMPOSITE POLYMER, WITH NANOSILVER OUTER LAYER AND BIODEGRADABLE INNER LAYER CONTAINING BIOACTIVE MOLECULES
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Objectives: Despite the myriad of skin substitutes, current gold standard treatment of full thickness burns remains split thickness autografts. However, their use cannot be extended to patients with a large affected %TBSA (Total Body Surface Area). The objective was to develop a scaffold for dermal replacement, based on new-generation nanocomposite materials.
Methods: A bilayered scaffold was developed; the biodegradable inner layer nanocomposite was 800µm thick and designed with a range of porosities, the 80µm outer, removable, non-biodegradable layer, contained nanosilver for antimicrobial protection. This scaffold underwent tensile testing, contact angle, permeability, FTIR and scanning electron microscopy (SEM) analysis.
Results: The physical construct was easy to handle and clinically applicable. Results demonstrate the macroporosity and permeability of the scaffold, which allowed 10mL min^{-1} of water to pass through; confirmed by SEM. Outer layer contact angle was over 90° and inner layer was <70° indicating hydrophilicity of the scaffold. Elasticity was clearly demonstrated by the Young's modulus.
Conclusions: This promising scaffold contains many desirable properties that could successfully mimic natural skin. Future directions involve covalently bonding bioactive molecules (i.e. cyclic RGD) and seeding the scaffold with adipose tissue-derived stem cells to enhance wound healing and angiogenesis.

P189 (EI0141)
PLASMA COATED ELECTROSPUN SUBSTRATES FOR MUSCLE TISSUE DEVELOPMENT
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Objectives: Epicardial implantation of an engineered muscle-graft has been associated with prolonged functional recovery of the ischemic area after myocardial infarction. However, highly organised and contractile *in vitro* muscle tissue development strongly depends on an appropriate design of the cell culture substrate. In the present study, the effect of substrate architecture and surface functionalization on muscle cell orientation, differentiation and contractility were investigated.
Methods: Aligned and randomly oriented micron- (3.2±0.8µm) or nano- (308±178nm) scaled fibrous polycaprolactone non-wovens were processed by electrospinning. A 15nm thick oxygen functional hydrocarbon coating was deposited at the surface by an RF plasma process (gas mixture: CO_2:C_2H_4 ratio 6:1; power input: 50 W; process duration: 20 minutes) and characterised by XPS. C2C12 muscle cells were grown on the substrates and analysed for viability, proliferation, orientation and differentiation. Cell orientation was characterised by a cosine function, where S=1 for parallel, S=0 for randomly oriented cells. Myotube maturation was analysed by immunofluorescence staining of sarcomeric striation. Contractile function was assessed under electrical stimulation.
Results: Plasma coating resulted in carboxylated, hydrophilic substrates. Cell viability varied from 40 to 60% relative to TCPS, with increased cell number on plasma coated substrates. Architectural cues highly influenced cell orientation. On aligned fibres, myoblasts displayed increased spatial orientation (S=0.91±0.03) as compared to randomly oriented fibres (S=0.33±0.2); p<0.01. Plasma coating however promoted differentiation, apparent by increased myotube formation. Accordingly, most pronounced sarcomeric striation was seen on plasma coated substrates where myotubes were furthermore most susceptible for electrical stimulation and resultant contraction.
Conclusions: The current study underlines the importance of combining architectural as well as chemical cues for advanced muscle development. Aligned fibres and plasma coating induce most pronounced myoblast differentiation. Furthermore, we provide evidence that a synthetic, fibrous substrate allows for myotube maturation and contractility.

P190 (EI0218)
CONTINUOUS MONITORING OF FEET TEMPERATURE IN PATIENTS WITH DIABETIC FOOT SYNDROME
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Objectives: Neuropathy of lower extremity is one of the complications of diabetes mellitus. The foot skin temperature is correlated with diabetic foot neuropathy. Monitoring of the foot skin temperature in diabetics may be a useful tool for preventing foot ulceration that in serious cases may result in foot amputation. The aim of our study was to determine daily course of temperature difference between ill (with a diabetic ulcer) and healthy foot.
Methods: Temperature measurement system developed in cooperation with the Center for Biomedical Technology (Krems, Austria) and Digilog Inc. (Perg, Austria) company was used for monitoring the skin temperature on feet. The device is able to gather 57 thousand of measurement data, which can be retrieved wirelessly using the RFID (Radio Frequency IDentification) technology. The diameter of the temperature sensor is 15mm, and its thickness is 6mm. The skin temperature measurements were performed every 1 or 5 minutes. Two healthy subjects and three diabetes type 2 patients were monitored for 1 – 6 days.
Results and Discussion: Assessment of the temperature differences was performed in 4 periods of day: 0:00-5:59, 6:00-11:59, 12:00-17:59, 18:00-23:59. The differences were lower than 0.2°C in healthy subjects. In diabetic patients those differences were significantly higher. The lowest temperature differences in all patients have been found during the night (0.67 °C to 1.02 °C), and the highest differences were registered in the afternoon (2.06 °C – 3.36 °C). Those findings are very important in the patient's state assessment.
Conclusions: The applied continuous temperature measurement system has

been found to be feasible. The obtained results indicated significant fluctuations of temperature differences between ill and healthy foot during the day. Thus, temperature as the marker of healing process has to be measured in carefully selected periods of the day.

P191 (EI0190)

RECONSTRUCTION OF EPIDERMAL AND DERMAL EQUIVALENT USING HUMAN KERATINOCYTES AND CHITOSAN SCAFFOLD CONTAINING HUMAN DERMAL FIBROBLASTS

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Objectives: In order to reconstruct dermal and epidermal equivalent using primary cultured normal human skin cells.
Methods: Normal human keratinocytes (HEK) and dermal fibroblasts (HDF) were isolated from foreskin by dispase II and sequential collagenase treatment. For epidermal equivalent, HEK seeded onto porous membrane was cultured and terminally differentiated by air exposure. For dermal reconstruction, chitosan scaffold was prepared by freeze-drying of 1.5% chitosan solutions. HDF was inoculated into chitosan scaffold, and cultured for 2 weeks.
Results: Three-dimensionally cultured epidermal equivalent was identical with normal human skin showing fully differentiated multilayer of basal layer, stratum spinosum, granular layer, and stratum corneum. Moreover, layer-specific marker expressions were also similar with human skin. Reconstructed dermal equivalent had similar physical characteristics with human dermis. It also showed porous structure of homogeneous chitosan matrix and well-spread HDF populations.
Conclusions: Reconstructions of epidermis and dermis were achieved respectively using normal human skin cells. Further efforts will be able to reconstruct the full-thickness skin equivalent using human skin cells and chitosan scaffold.

P192 (EI0183)

THREE-DIMENSIONALLY CULTURED HUMAN NASAL EPITHELIAL CELL SHEETS FOR TOXICITY TESTS AND CLINICAL APPLICATIONS

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Objectives: In order to establish the *in vitro* cultured human nasal epithelial reconstruct for alternative toxicity test and even clinical applications.
Methods: Normal human nasal epithelial cells (HNE) were isolated by dispase II treatment, and inoculated onto porous membrane. The cells were cultured under submerged until confluent, and sequentially differentiated by air exposure. Fully differentiated cell sheets were compared with intact human nasal epithelium by H&E and immunohistochemical staining. For further clinical applications, HNE were seeded onto human amniotic membrane, and its histological characteristics were also compared with human nasal epithelium.
Results: Primary cultured HNE showed cobblestone-like morphology without contamination with other cell types. Histological observation showed well differentiated cilia. Immunohistochemical staining revealed the expression of p63 at basal cell nucleus, CD44v6 at cell-to-cell junctions of basal cell layer, Na^+/K^+ ATPase at apical layer. In addition, CoIIV, LN5, CK3/12, CK13, CK5, MUC1, and MUC5AC were positively expressed. Moreover, 3-dimensionally cultured human nasal epithelial cells onto human amniotic membrane showed identical histology and marker expressions with native human nasal epithelium.
Conclusions: *In vitro* differentiated nasal epithelial cell sheets were not only suitable for alternative toxicity test due to its similarity with nasal epithelium, but also available for clinical applications.

P193 (EI0262)

TISSUE ENGINEERING OF SKIN: BIODEGRADABLE SCAFFOLDS INCORPORATED INTO LABORATORY-GROWN SKIN SUBSTITUTES

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Objectives: Extensive skin loss, such as the skin defects associated with deep burns, avulsion injuries, or giant nevi, still represents a significant clinical problem. A promising approach to treat large skin defects may be the use of a tissue engineered full thickness skin analogue with near normal anatomy and function. Apart from the crucial biological properties, such a skin substitute should exhibit adequate structural features, particularly tensile strength and optimal pliability. The goal of this study was to test whether two polymeric net-like fabrics, one already clinically established and the other one consisting of a well-known clinically established material, can be used for skin tissue engineering.
Methods: Both scaffolds were integrated into a collagen type I hydrogel and dermo-epidermal skin substitutes were generated. The skin substitutes were transplanted onto immuno-incompetent rats and analyzed three weeks thereafter, employing histological analysis, immunofluorescence and scanning electron microscopy.
Results: We found that these substitutes exhibited a well stratified epidermis that had homogeneously developed over the entire surface of the grafts. This epidermis had deposited a functional basement membrane, displayed a basal cell layer (stratum basale), several suprabasal layers and a stratum corneum. Additionally, the new skin was well vascularized even around the remnants of the supporting net-like polymer.
Conclusions: We consider these novel dermo-epidermal skin substitutes as very promising skin analogues for the treatment of full thickness skin defect.

P194 (EI0066)

COMPARATIVE ANALYSIS BETWEEN ACELLULARIZED AND IMMUNOLOGICALLY NON-TREATED VASCULAR XENOGRAFTS IN LONG-TERM SURVIVAL ANIMALS

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Objectives: We implanted acellularized and immunologically non-treated porcine xenografts as an arterial graft in goats and comparatively analyzed the explanted grafts with gross observation, as well as light microscopy and immunohistochemistry, following the predetermined periods.
Methods: For immunologically non-treated xenografts, bilateral porcine carotid arteries were harvested, and after short-term freezing at -70°C, were implanted into goats. The preparation of acellularized xenograft vessels has been performed with Nacl-SDS solution and stored at the freezer until use. The goats were randomly assigned for five periods of observation (1 week, and 1, 3, 6, and 12 months after implantation), four animals were observed at each of these times. Periodic ultrasonographic examinations were performed during the observation period. Following the predetermined periods, the explanted grafts were analyzed.
Results: Among 20 animals, two goats died prematurely, and a total of 35 grafts were evaluated. Gross observations revealed nonthrombotic patent smooth lumens. Microscopic examinations of the explanted grafts showed satisfactory cellular reconstruction up to the 12-month observation period. The proportions of CD3 positive T lymphocytes among inflammatory cells infiltrations were very low.
Conclusions: In conclusion, these findings, as a whole, suggest that porcine vessel xenografts can be clinically acceptably implanted in the goats as a form of small-diameter vascular graft, regardless of the acellularized xenograft or immunologically non-treated xenograft.

P195 (EI0001)

NEW CARDIAC BIOREACTOR FOR MECHANICAL CONDITIONING OF TISSUE-ENGINEERED VALVULAR AND VASCULAR SUBSTITUTES

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The pathologies observed after the implantation of currently available aortic valve substitutes, such as thrombosis or accelerated tissue degradation, call for the development of an improved type of substitutes. Tissue engineering can provide, using cell culture techniques, aortic valve substitutes free of these problems, with the ability to grow, repair and remodel. In order to confer proper mechanical and biological properties to the tissue-engineered substitutes, cells must be cultured in an environment recreating hemodynamic conditions, hence the need for bioreactors. The objective pursued with the development of our new cardiac bioreactor is to reproduce, with the highest level of accuracy, any blood flow and pressure conditions up to the physiological range. The design of the bioreactor is based on a two-element Windkessel model. A diaphragm pump, driven by compressed air, is used to generate a pulsed flow within the system. A compliance chamber and resistances, for which the parameters are manually set, are used to passively modulate the pressure waveform generated. Manual optimization of the user-defined command to the system allowed reproducing physiological flow and related pressure waveforms with very good corre-

lation. However, better accuracy may be achieved by closing the control loop of the system. The aim of the next prototype, which is actually under development, is then to enhance control over the reproduction of the hemodynamic conditions. An automated active component is being designed, in order to replace the compliance chamber and the resistances in their role to modulate pressure by actively modifying the behavior of the system during each beat. Controllers using artificial intelligence algorithms, such as neural networks, are implemented to automatically control the actuation parameters of the diaphragm pump and automated component.

Latest advances in preventive and regenerative medicine technologies

P196 (EI0351)

THE DEVELOPMENT OF AN ARTIFICIAL TRACHEA

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Objectives: Our team at UCL has recently performed the first stem cell organ transplant in the world of a fully tissue-engineered airway with a hugely successful outcome. For this case, a human donor trachea was harvested, decellularized and reseeded with the patient's own epithelial and mesenchymal stem cells (MSCs). The aim of this current study is to continue this research but with the use of an artificial tracheal replacement instead of delcellularized human tissue. This artificial trachea is composed of a POSS PCU polymer which has been developed in our laboratory and has undergone extensive testing, proving its huge potential for use in surgical implants.
Methods: The biocompatibility, non-toxicity, low inflammatory response and inert nature of the polymer were established by implanting the polymer in a sheep model for 36 months. The cytocompatibility of the polymer was shown by seeding MSCs and epithelial cells onto the polymer and monitoring their attachment and proliferation using scanning electron microscopy, immunostaining and histological analysis. The structural design of the construct and specific fabrication of the polymer was determined using numerical modelling.
Results and Discussion: The construct has proven to be biocompatible, non-toxic, have a low inflammatory response and support the attachment and proliferation of tracheal specific cell types. In addition the construct avoids luminal collapse and retains sutures.
Conclusions: A tracheal replacement has been developed, which mimics the structure and function of the native trachea. This artificial trachea could be the first step in the use of 'off the shelf' technology leading to the potential elimination of the current organ donor shortage problem.

P197 (EI0320)

EXAMINING ENDOTHELIAL PROGENITOR CELLS (EPCS) FOR TISSUE ENGINEERING PURPOSES WITH A NEW SHEAR STRESS DEVICE

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Objectives: Recent findings indicate that Endothelial Progenitor Cells (EPCs) can accelerate re-endothelialization of vessels. An intact endothelium is essential for hemocompatibility and prevention of thrombi formation. Thus, these cells are about to become an important tool for tissue engineering of grafts that have contact with blood. In our new bioreactor system we exposed late outgrowth EPCs from human peripheral blood to defined shear stress conditions and examined their behavior.
Methods: We developed a novel bioreactor system that provides defined levels of shear stress. Basically the device consists of two coaxial cylinders with different sizes. The inner one rotates and impels medium in the gap between both cylinders, which results in shear stress for the cells on the inside of the outer cylinder. Ports permit sterile withdrawal and injection of fluids during the run. Medium temperature is checked by incorporated sensors and the attached microscope system allows monitoring of cells. Expression of endothelial specific proteins PECAM-1 and VEGF-R2 was evaluated by immunocytochemistry and quantitative real-time PCR.
Results: Using our device we were able to show differences in the expression of endothelial cell proteins in EPCs depending on the applied shear stress level. Time lapse recording of the cells not only depicted how EPCs detached during flow experiments but also revealed interesting changes in their morphology. Alteration of the cells was also prominent at mRNA transcription and protein expression levels.
Conclusions: Our bioreactor system is a feasible device for the investigation of adherent cells under defined shear stress conditions. Interestingly, the forces of the fluid flow had influence on EPCs which was not only visible in morphological changes of cells but also at transcription and protein expression levels. Therefore more tests with EPCs will be necessary to finally figure out their suitability for tissue engineering purposes.

P198 (EI0285)

DIRECT *IN VIVO* OBSERVATION OF LEAFLET TISSUE FORMATION FOR BIOVALVE

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Objectives: We have developed the autologous tissue heart-valved conduit "Biovalves" grown in the recipients' subcutaneous spaces, which were autonomically formed precisely according to the shape of the material molds by encapsulation with connective tissues. In this study, the formation process of the Biovalve leaflet tissue was directly observed by wireless video capsule endoscope.
Methods and Results: The mold for Biovalve preparation was consisted of five main plastic parts. The wireless video capsule endoscope was completely impregnated in one plastic part for the conduit (diameter: 16mm). The assembled molds were placed into the dorsal subcutaneous pouches of goats. The endoscope could clearly observe the small aperture (0.5~1.5mm) prepared between the conduit part and leaflet part with protrusion resembling the sinus of Valsalva for leaflet formation. The observation was performed every day during tissue formation for about 1 month. The air collected at the aperture at the placement had disappeared within 2 weeks. Until this time thin white solid fulfill the aperture completely. The connective tissue ingrowth started from the edge of the aperture 2 weeks after the placement. The forefront of the tissue formation accompanied with a lot of capillary. The condense tissue gradually migrated to the aperture. At about 1 month the aperture was completely covered with the tissue to form leaflet tissue. Upon removing the molds from the harvested implants Biovalve with robust collagenous leaflets were obtained.
Conclusions: The leaflet tissue formation could be clearly observed by using wireless video capsule endoscope-embedded molds, which is one of the major steps toward its clinical application.

P199 (EI0258)

THE VALUE OF RESISTANCE INDEX (RI) IN PROGRESSION OF DIABETIC NEPHROPATHY AND CHRONIC RENAL FAILURE

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Objectives: Intrarenal resistive index (RI) demonstrates changes of renal vascular resistance and determines evolution in patients with diabetic nephropathy.
Methods: Intrarenal RI values were achieved from intraparenchimal arteries; values >0.70 are considered pathologic. The study was longitudinal. Clinical parameters and renal function were evaluated at baseline and after 3, 6, 9, 12, 15, 18, 21 and 24 months. 70 patients with diabetic nephropathy were divided based on their intrarenal RI: group 1 had values of ≥70, group 2 had values <70. A group of 30 healthy volunteers, matched for age, sex and body mass index, was used as control.
Results: Intrarenal RI value ≥70 had 64.3%, at baseline; 50% of them had a decline in renal function after 9 months and 64% after 24 months. In patients with intrarenal RI values <70, 34% had a decline in renal function after 24 months. In multivariate regression analysis, proteinuria, higher baseline Ccr and RI were independent predictors of declining renal function. RI values were significantly affected by mean blood pressure, DeltaCCr and proteinuria. The relationship between the RI values and CCr (Delta CCr) showed a negative correlation coefficient of r=-0.630 (P<0.01). There was no relationship between CCr and age

and RI and age in diabetic patients.
Conclusions: The RI can be used as a non-invasive, easily available parameter of the evolution in patients with advanced clinical diabetic nephropathy. An intrarenal RI value of ≥70 identifies diabetic patients at risk for progressive renal disease.

P200 (EI0221)
HEPATIC METABOLISM OF ENCAPSULATED PRIMARY HUMAN PROGENITORS AND HEPATOCYTES
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Objectives: Some diseases such as drug diseases or hepatitis may affect hepatic functions and liver transplantation is often the only treatment available. To overcome the shortage in transplantable liver, implantation of primary human hepatocytes isolated from non transplantable livers can be proposed in some cases. One major limiting factor of the application is the significant cellular loss after transplantation. A new approach based on the encapsulation of primary human hepatocytes before implantation is a potential solution to enhance the engraftment of the implanted cells. It is shown in the literature that 3D culture conditions maintain the metabolic functions of mice and rat primary hepatocytes and enhance the differentiation of hepatic progenitors. The purpose of this project is to study the differentiation ability and the metabolic maintenance of the encapsulated primary human progenitors and hepatocytes under 3D culture in porous beads.
Methods: Primary human progenitors and hepatocytes were obtained from surgical liver resections. The isolated cells were encapsulated in alginate and collagen beads combined or not with Poly-L-Lysine. The beads were produced using a co-axial air flow extruder (home-made design). The cells, encapsulated or not, were cultured in adapted media and their metabolism was studied at different time points.
Results and Discussion: The metabolic functions and differentiation ability of the encapsulated cells were compared to the results obtained in 2D culture of collagen coating. Moreover, to improve the biocompatibility of the beads, a layer of Poly-L-Lysine was added on the surface of the beads. We also studied the effect of this layer on the encapsulated cell functions.
Conclusions: Several configurations will be implanted in a rodent model, in order to reinforce the feasibility of the approach. Specific experiments will be developed to localize the position of the cells hosting beads.

P201 (EI0201)
MEASLES VIRUS PRODUCTION PROCESS FOR THE USE IN CANCER THERAPY
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Objective: Measles virus which is adapted to tissue culture showed selective tumor cell killing abilities with attenuated pathogenicity. According to the dose needed for measles vaccination in cancer therapy a tenfold higher amount of the virus is needed. In this work, the production of large quantities of measles virus particles in a standardized manufacturing process should be established. First results of the work are presented here.
Methods: Measles virus production was carried out in spinner system at 37° C, 40rpm and 5% CO_2. The offline tracking of cell growth was carried out by fluorescence-based and activity-based assays. The measles virus concentration was estimated by the $TCID_{50}$ method.
Results: The cells have been adapted to a commercially available serum-free medium. In addition, all products of animal origin have been replaced. The growth surface is provided by micro carriers for the cultivation of adherent cells in stirred systems. Appropriate micro carriers were selected by comparing the adhesion capacity of the cells, the growth and the glucose consumption rates. Virus production rates showed similar results for cell associated virus and from the supernatant. In addition, research on the temperature stability of the virus was carried out. It has been shown that the virus in the supernatant under culture conditions was very unstable.
Conclusion: It is a common belief that most of the virus amount remains cell-associated. This data showed new results by getting much larger amount of virus by harvesting the virus continuously from the supernatant. To meet commercial and regulatory requirements, this process must be high yielding, scalable and reproducible. These requirements are met by establishing a cell culture process employing stirred system and serum-free cell culture medium. The first results are promising for a successful scale up in the bioreactor.

P202 (EI0078)
ESTER OF HYALURONIC AND BUTYRIC ACID PROTECT RENAL CELLS IN AN *IN VITRO* MODEL OF ISCHEMIC INJURY
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Objectives: Acute Kidney Injury (AKI) is a rapid decline in renal function characterized by acute tubular necrosis, but also by mesangial cells (MC) proliferation and matrix deposition that represent elements of graveness and progression toward fibrosis. Recently we demonstrated in a rat model of AKI a higher improvement of renal function using mesenchymal stem cells pretreated with esters of Hyaluronic and Butiric acids (HB), compared to untreated cells. Here we investigate if HB can prevent MC proliferation, reduce matrix genes expression and prevent cell death in an *in vitro* model of oxidative stress, one of the main causes of AKI.
Methods: Rat MC were pretreated with HB (1g/L) or grown in culture media for 24h, before adding H_2O_2 50μM for 6-16-24-48h to induce an oxidative damage. MC proliferation was assessed by cell cycle analysis; MMP-9 and collagen-1 gene expression was evaluated by Real-Time PCR. Apoptosis and necrosis were assessed by analysis of caspase-3 activity and lactate dehydrogenase release. Investigation of involved pathways (Akt and p38) was performed by Western Blot.
Results: At 24h, the 35% of H_2O_2-group is in G2/S phase, compared to 10% of control and 23% of HB-group. The reduction of proliferation is joined by a significant decrease in Akt phosphorylation. HB induces a significant increase of MMP-9, involved in matrix degradation, at every time considered, and a reduction of Collagen-1 expression at 24h, compared to H_2O_2-group. H_2O_2 treatment causes necrosis, that is strongly inhibited by HB. At 16h caspase-3 activity is higher in HB group, indicating that in this case cells die for apoptosis, as confirmed by P38 phosphorylation, preventing the inflammation necrosis-related.
Conclusions: We demonstrated that HB protect MC from injuries induced by oxidative stress, suggesting its employment as first aid to rescue a damaged kidney, that may be followed by delayed transplantation of stem cells.

New Biomaterials and Scaffolds

P203 (EI0386)
***IN VITRO* MAGNESIUM DEGRADATION WITH VARIATION OF FLOW RATE AND PRESSURE**
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The use of magnesium as a bioresorbable material for clinical applications is very interesting because of his mechanical and biocompatibility properties. Magnesium is not in use in medical applications, because the degradation mechanism and the mechanism of some observed effects are not yet fully understood. A review revealed that the comparison of literature data is not possible, because many studies deal with different methods like static, electro chemic and some dynamic techniques with a wide range of different test fluids and test parameters.
Objectives: The effects of the magnesium degradation and its degradation in a biological environment are not yet understood. The focus of this project is to investigate the degradation mechanism of these effects and to improve the degradation behaviour of magnesium for implantation purposes.
Methods and Results: With the objective of the standardization of degradation studies a dynamic *in vitro* test system was developed. Temperature, flow rate, and pressure of the fluid can be controlled in this system in order to investigate their influence on the degradation process. Within ongoing studies, the dynamic degradation system depicts the influence of flow rate and static pressure on the degradation process in distilled water and saline solution. The degradation process is analyzed by measuring the mass loss, the hydrogen release and the Mg^{2+} concentration in the solution.

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Conclusion: Since the magnesium degradation process and the interaction between the biological environment is not exactly understood, the first step towards standardization of degradation studies has been done by classifying the influence of the flow rate and the pressure on the degradation rate. In further studies various model fluids, which are described in the literature, will be tested in this new set-up.
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P204 (EI0332)
USING LACUNARITY TO CHARACTERIZE PORE DISTRIBUTION IN SCAFFOLDS
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Objectives: The performance of scaffolds for cell growth is largely influenced by their physical properties. In particular, it is well known that porosity plays a major role. However, the characterization of the spatial distribution of pores in irregular scaffolds is a challenging task, when the pores distribution is not homogeneous, with pores either clustered or dispersed. Here we propose lacunarity (LAC) as a metric suitable for the characterization of the spatial distribution of pores in scaffolds for tissue engineering.
Methods: Submicron resolution microCT images of a commercial scaffold (Chondro-Gide, CG) were used. From them, synthetic images with the same porosity as the CG, but with different spatial distributions of pores, were generated. On both recorded and synthetic images LAC was calculated, in order to measure the spatial distribution of pores. Lacunarity analysis is a multi-scaled method of determining the texture associated with patterns of spatial dispersion. Lacunarity provides an analysis of scaffold images in terms of 1) the overall fraction covered by the attribute of interest, 2) the presence and scale of randomness and 3) the existence of hierarchical structure.
Results: WE observed that in scaffolds with the same macroscopic porosity value the greater the degree of pore clusterization, the higher the value of LAC. In fact, in synthetic scaffold images with porosity equal to 50% and with high clusterization, LAC is 1.83, while in the presence of dispersed pores LAC is reduced up to about the 25%. Interestingly, we conjecture that the CG is characterized by dispersed pores, because we calculated a LAC equal to 1.38 on its microCT images.
Conclusions: Our findings clearly show that LAC can be a powerful tool for scaffolds properties characterization. In the future, pore clusterization will be analyzed to get insight into its influence on physical parameters such as permeability.

P205 (EI0212)
BLOOD PERMEABILITY EXAMINATION OF DIFFERENT SEALING MATERIALS FOR POLYESTER VASCULAR PROSTHESIS
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Objectives: The aim of this study was the determination and comparison of blood permeability of different sealing materials for vascular grafts.
Methods: The investigation materials were sealings based on microcrystalline chitosan, PHB or albumin-dextran mixtures applied on Bard 004187 vascular grafts. As an operating medium antycoagulated CPDA-1 porcine blood was utilized. Innovative measurement methods of blood permeability through vascular grafts in relation to the standard ISO 7198:1998(E) were designed. A static test was performed for all sealing materials. The dynamic test was fulfilled for sealings with the lowest permeability. The static test consisted in generating hydrostatic pressure of 100mmHg in the sample by putting the blood reservoir 1.2m above the sample. The dynamic test consisted on placing the graft samples in a mock circulatory system were pulstile pressure of 110±60mmHg was generated. The time and the amount of collected blood were determined.
Results: For each sealing type different compositions of the sealing layer were examined. For six microcrystalline chitosan sealings the blood loss varied from 0.7 to 60.7 mL/min/cm^2 and for nine PHB sealings varied from 50.69 to 613 mL/min/cm^2. Tree albumin-dextran sealings showed no blood lost. In the dynamic test the albumin-dextran sealings showed a blood lost less than 0.01 mL/min/cm^2.
Conclusions: No sealing properties were observed in relation to PHB derivates sealings. Different sealing potential characterized microcrystalline chitosan sealings. The best sealing effect was observed for vascular grafts sealed with dextran-albumin mixtures.

P206 (EI0193)
RECENT RESULTS OF NOVEL SILICONE HOLLOW SPHERE FIBERS (SHSF) FOR MEMBRANE OXYGENATOR MODULES
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Objectives: Major limitation of conventional silicone hollow fibers (HF) once compared with micro-porous HF is poor gas permeability. However, unpredictability of plasma leakage, foam formation, and brittleness elevate risks of blood trauma, thus restricting long-term application of micro-porous fibers in ECMO. Limitation of the poor gas permeability in silicone is difficult to overcome by fabricating fine, thin hollow fibers for the reduction of resistance. We introduce a novel type of pure diffusive silicone capillaries (SHSF) with walls embedding micro spheres: by enclosure of such micro spheres, a high gas exchange performance is established due to resistance reduction, in addition to adequate stability by preserving the membrane wall thickness.
Methods: SHSF are cross-wound into cylindrical silicone flexible housing. SHSF (RAUMEDIC®, AG) with 200-400µm and 100µm wall thickness embracing 40% ratio of embedded air spheres to impose an optimal compromise between structural stability, and gas exchange efficacy. Small-scaled oxygenator-modules for low flow rates using SHSF, as well as conventional HF as control modules were constructed for *in vitro* validation in a custom-built experimental circuit.
Results: 11 different module types, besides 2 control modules made of conventional micro-porous fibers were constructed**.** As an example: the smallest module has a priming volume of 1.6mL. It contains 200 fibers, and an effective length of 125mm. Membrane surface area amounts 0.02m^2. Water as fluid phase is used in order to preserve fibers for subsequent reproducible testing. The inlet PO_2 value of 70mmHg can be oxygenated up to 430mmHg, leading to an elimination PCO_2 difference of 16 mmHg at a flow rate of 50 ml/min. Preliminary results with whole blood demonstrate the modules efficiency.
Conclusions: Results obtained indicate that SHSFs represent a promising alternative to both conventional micro-porous and silicone HF. Further experiments with blood will be the next step for more tangible validation of efficacy.

P207 (EI0082)
COMPOSITES OF HYDROXYAPATITE DOPED WITH NANO-POWDER OF TITANIUM OXIDE
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Objectives: Hydroxyapatite (HA) is currently the most demanded biomaterial for reconstructing human skeleton. Ceramics based on HA have been successfully used for many decades in medicine. Nevertheless, the poor mechanical properties of HA prohibit HA application in the areas of the skeleton which undergo high mechanical loading. The mechanically weak HA can be reinforced with different oxides after sintering. Usually micro-sized reinforcement materials have been used but there are few publications reporting the addition of nano-sized reinforcement materials. Since earlier studies showed that Ti containing composites are highly biocompatible in cell cultures, this study determined the structure and the mechanical properties of bovine derived hydroxyapatite (BHA) doped with nano-powder of TiO2.
Methods: Composites of calcinated BHA and nano-powder of TiO2 were prepared by adding 5 and 10wt% of TiO2 in BHA powder. The powders mixtures were homogenized with ball milling, pressed into pellets, and finally sintered at different temperatures between 1000°C and 1300°C for 4-hours in air. Microstructure observations with SEM and density measurements were carried out along with compression strength and microhardness tests.

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Results: The sintered samples comprised HA and Ti-oxide associated phases. The compression strength and the microhardness of the HA-TiO_2 composites increased with increasing TiO_2 content and sintering temperature (up to 1300°C); the highest values were 139.95MPa and 316.5HV, respectively (for 10%TiO_2 and 1300°C).
Conclusions: The high mechanical properties of the produced composites in conjunction with microstructure features and in the light of their earlier reported biocompatibility, qualify them further consideration in developing promising new bioceramics.

P208 (EI0098)
PRODUCTION OF BIOCERAMICS NANO-PARTICLES FROM EGG SHELLS WASTE
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Objectives: Eggshells approximately comprise 96wt.% mineralized phases, which predominantly consists of $CaCO_3$, and 4wt.% organic matter; minor amounts of other oxides also exist. It is very easy to collect eggshells from home, bakery shops, food factories, etc. Eggshells can be regarded as a very easy and pure source for hydroxyapatite (HA) production via various hydrothermal processing methods. The aim of this study was to produce nano-particles of HA via other simple methods, specifically ultrasonic and hotplate methods.
Methods: Eggshells were collected and cleaned by deionized water from organic matter and dried. They were subjected to ball milling until powder of 100µm was obtained. The powder was subjected to DTA analysis to determine the content of $CaCO_3$ and thereby the equivalent amount of phosphoric acid needed to be added to form TCP and HA via ultrasonic and hotplate methods. The obtained powders were sintered at 450°C and 850°C for 4 hours in air. The sintered bodies were characterized with IR and SEM analysis.
Results: Ultrasonic agitation stimulated the reactivity of chemical species through particle size reduction and surface activation by intensive stirring, resulting in the acceleration of the heterogeneous reactions between liquid and solid reactants effectively and taking extracts from liquid phase. Hotplate method enabled HA formation. X-ray diffractograms indicated various phases of TCP and HA formed after 4-hour sintering at 450°C and 850°C. **Conclusions:** Ultrasonic and hotplate methods feature safety and versatility with regards to fabricating HA and other similar phases. The low cost and the possibility to produce nano-powders of HA and TCP are two more very attractive advantages of these techniques.

P209 (EI0094)
MICROSTRUCTURE AND MECHANICAL PROPERTIES OF SINTERED SHEEP ENAMEL-DERIVED HYDROXYAPATITE
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Objectives: Calcium phosphate ceramics are popular materials for bone reconstruction and reinforcement for a long time. Hydroxyapatite (HA), one of the calcium phosphate ceramics, has been successfully applied in medicine due to its excellent biocompatibility with hard tissues. Since its chemical and crystallographic properties closely resemble those of bone and tooth minerals, HA attracts a particular interest for bone grafting, augmentation in maxillofacial surgery and in orthopedics as space filling material. There is limited literature information on using bovine enamel as graft material. Moreover, there are still no attempts to use sheep enamel (and dentine) HA as a graft material. The aim of this study was to investigate the structure and the mechanical properties of bioceramics from sheep derived enamel HA.
Methods: Bovine teeth were collected and cleaned from fatty tissues. Then they were subjected to calcination at ca. 850°C. Enamel and dentine parts were separated easily. Enamel parts were wet ball milled until fine powder (100µm) was produced. The dried powders were dry pressed to cylindrical green samples, suitable for compression test. The samples were sintered for 4-hours in air at several temperatures between 1000°C-1300°C. Microhardness, compression strength and density measurements along with X-ray diffraction analysis and SEM observations of the sintered samples and statistical-tests were realized.
Results: The best values for compression strength and microhardness were obtained for the samples sintered at 1300°C, namely 100.17MPa and 366.36HV, respectively. These results agree fairly well with the density measurements as well as the crystallographic analysis and the microstructure observations.
Conclusions: The comparison of the results of this study with those obtained from earlier studies where HA was derived from bovine bones (BHA) or BHA composites shows that the sheep derived enamel HA results in superb biomaterials. Moreover, this study proposes a production of HA from an economic and natural source.

P210 (EI0091)
HYDROXYAPATITE PRODUCTION WITH VARIOUS TECHNIQUES FROM SEA URCHIN
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Objectives: Natural species of sea origin, such as corals and nacres, always attract special interest in biomaterials science and technology because of excellent biological properties. Their bio-mineralization mechanism has been extensively investigated and documented. Nevertheless, there are only few studies dealing with the use of sea urchins as biomaterials. The aim of this study was to fabricate various types of biological active hydroxyapatite (HA) nano-particles with various methods.
Methods: Sea urchin skeletons were collected from beaches of Marmara Sea and then brushed, washed, and dried. The particles were milled until fine powder of 100µm was produced. DTA analysis results were used to calculate the equivalent amount of phosphoric acid needed to satisfy the stoichiometry of HA. The collected powder was rinsed into distilled water. For the hydrothermal transformation, the suspension was put separately to ultrasonic bath and hotplate. After 15 minutes, the equivalent phosphoric acid was added drop by drop. The treatment was continued (either in the ultrasonic bath or in the hotplate) for 2 more hours. Then, the precipitates were removed from the suspension. After drying, the powders were sintered for 4 hours in air at 450°C and 850°C. The sintered bodies were analyzed with FTIR, X-ray diffraction, and SEM.
Results: The sintered bodies contained various phases of HA and TCP, as revealed from the XRD and IR analysis. Fine microstructures of nano-sized grains were observed with SEM.
Conclusions: This study presented easy production methods of HA with hotplate and ultrasonic. Thus, conventional hydrothermal methods, such as those that employ high-pressure vessels, which could be very dangerous, tedious and expensive, can be omitted.
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P211 (EI0074)
MICROSTRUCTURE AND MECHANICAL PROPERTIES OF COMPOSITES OF BOVINE DERIVED HYDROXYAPATITE (BHA) DOPED WITH NANO-POWDER OF LANTHANUM OXIDE
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Objectives: Hydroxyapatite (HA) is one of the most widely used biomaterial

for skeleton reconstruction since decades. HA materials are very popular for bone restorations because they accelerate bone growth around the implant due to their chemical and crystallographic similarity to human carbonate apatite. Nevertheless, HA ceramics are not used in load carrying applications because of their poor mechanical properties. To improve the mechanical properties of HA-bioceramics, such as microhardness and compression strength, metallic materials, various ceramic oxides, or whiskers, can be added in HA for fabricating composites. The aim of this study was to investigate the structure and the mechanical properties of composites of bovine derived HA (BHA) doped with nano-powders of lanthanum oxide.
Methods: BHA was mixed separately with 5 and 10wt% nano-powder of lanthanum oxide with dry ball milling for 4 hours. Green bodies of cold-pressed samples were sintered in air for 4 hours at different temperatures (1000–1300°C). Density, microhardness, compression strength, X-ray diffraction and SEM observations were conducted.
Results: The best values of compression strength and microhardness were obtained for the samples sintered at 1300°C (specifically 130.75MPa and 385.54HV for 5% doping and 123.28MPa and 434.45HV for 10% doping). These results are consistent with the density measurements, the results of X-ray diffraction analysis and the SEM observations.
Conclusions: Provided that small amounts of lanthanum can be safely incorporated in biomaterials without jeopardizing bioactivity, the addition of the nano-powder of lanthanum oxide in the matrix of biological HA seems to result in a composite structure, which is resistible against loads
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Modelling in the Cardiovascular System

P212 (EI0318)
A VARIABLE ELASTANCE-BASED MOCK CIRCULATION MODEL FOR REPLICATING HUMAN CARDIOVASCULAR SYSTEM
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Objectives: The objective of the paper is to develop a mock circulation loop which can represent (i) the left and right parts of the heart and (ii) systemic and pulmonary vascular branches. The loop has variable elastance based left and right ventricular actuation. The system will be configurable to model congestive heart failure for testing of left, right and bi-directional ventricular assist devices (LVAD, RVAD, Bi-VAD).
Methods: A software based cardiovascular model is run in parallel with the mock circulation. Left and right ventricular volumes and elastances are supplied as reference to form the cardiovascular model to the loop. Hydraulic pumps will use them to repeat the pulsatility of the ventricles. The other compliances will be modelled by passive closed to atmosphere (arterial) windkessels and open (atrial and venous) chambers. Vascular resistances will be modelled by pinch valves.
Results: Two sets of results will be presented; these are the pressures, ventricular volumes and systemic arterial flows of (i) healthy human dynamics and (ii) dynamics for end-stage congestive heart failure. These reflect the changes in system parameters of ventricular elastances, vascular resistances and systemic arterial compliances from healthy to ill mock circulations.
Conclusions: A mock circulation is developed to replicate healthy and ill dynamics of a cardiovascular system. The system has more realistic presure and volume signals as a result of variable elastance based excitation from a cardiovaascular model.

P213 (EI0266)
HIGHLY TRANSPARENT HOLLOW MODELS FOR FLOW VISUALIZATION - A SWEET SECRET
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Objectives: In order to visualize the flow and interaction between cardiovascular structures and prostheses, transparent models with excellent optical qualities are necessary. Such models are mostly made from casts starting with positive prototypes created by CNC-milling or 3D-printing. To get the final transparent model an intermediate dead-mould from an easily removable material is required. This material must allow casting of perfectly smooth surfaces and must not interact with the surface of the final silicone material. Usually, for this purpose either wax or low-melting metal is used. However, wax tends to penetrate the silicone and so destruct the surface. Low-melting metals are expensive or toxic. We developed a method, based on chocolate.
Methods: To create models of the aorta, first a positive prototype of the inner aortic surface was printed on a 3D-printer (Eden350 Objet-Geometries Ltd., Israel, Material: FullCure720). This model was then molded with a silicone negative form, which was devided into two halves. After removing the positive prototype, the silicone-casting mold was filled with baking chocolate. The chocolate aorta was coated with colored polyvinyl alcohol and then casted with highly transparent silicone (Sylgard184, Dow-Corning Corp. MI, USA). After curing, the chocolate was melt out by hot water.
Results: The created models were of perfect transparent quality for PIV with 20um-particles and for videotaping valve interactions. Eventual modifications could be easily performed. With a refractory-adapted blood mimicking fluid (glycerine/water/Na-jodide) an adaption up to even invisible boundary surfaces could be achieved.
Conclusions: The described process is a cheap and effective way to create transparent models with excellent optical quality.

P214 (EI0170)
LIPOSOME-ENCAPSULATED HEMOGLOBIN AMELIORATES BRONCHIAL ANASTOMOTIC HEALING AFTER RADIATION AND PNEUMONECTOMY IN THE RAT
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Objectives: Liposome-encapsulated hemoglobin (LEH), an artificial oxygen carrier, has been reported to accelerate surgical wound healing. In the current study, LEH was tested in bronchial anastomotic healing after radiation and pneumonectomy as an experimental model of combined treatment for lung cancer.
Methods: SD rats received preoperative radiation (20 Gy) to the chest as a simulation of cancer radiotherapy. Four days later, all animals underwent the left pneumonectomy with the bronchial stump closure (Sweet method) using 3 stitches of 7-0 monofilament suture. At the operation, rats were randomly assigned to receive intravenous infusion of LEH with high O_2 affinity ($P_{50}O_2$=17mmHg, 10 ml/kg, n=17) or saline as a control (10mL/kg, n=19). Additional rats (n=33) were treated in the same way without preoperative radiation. Bronchial anastomotic healing was evaluated 2 days after surgery, when the animals were sacrificed to determine bursting pressure of the bronchial suture line.
Results: In rats with no preoperative radiation, bursting pressure was equivalent 2 days after pneumonectomy regardless of LEH-treatment (154±94mmHg, n=17) or saline control (138±81mmHg, n=16). In rats pretreated with radiation, however, LEH was significantly more effective in preserving the bursting pressure (150±42mmHg, n=17, *P*=0.044) than in radiated control rats receiving saline (110±61mmHg, n=12).
Conclusions: While the effect of LEH (10mL/kg) with high O_2 affinity ($P_{50}O_2$=17mmHg) was not significant in untreated rats, mechanical strength in bronchial anastomosis was significantly preserved by LEH-treatment in rats pretreated with radiation. The results suggest that LEH may be protective of bronchial anastomotic healing in compromised host by preoperative radiation therapy.

P215 (EI0254)
PURPOSE OF PARALLELIZING CANNULAS OF OBESE PATIENTS ON ECMO SUPPORT
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Objectives: Obese patients have a significantly higher risk of stroke complications during ECMO support. That risk is connected with advanced state of atherosclerosis. Larger patients need higher perfusion flow, which needs high pressure in the ECMO set. Then, the flow from outflow cannula is of high energy, which could result in releasing of atherosclerotic plaques.
Methods: The best way to lower the systemic pressure is to lower the resistance of junction between patient and the ECMO system. This resistance is formed on

inflow/outflow cannula. Inspired from electrical analogies, we tried to lower the pressure by parallelizing the inflow and outflow cannulas. We designed a simple model to illustrate this approach.
Results: In ideal cases, the pressure is two times lower with paralel connection than with standard connection. This positively influences the blood energy in outflow cannula and lowers the risk of releasing atherosclerotic plaques. We are aware of complications associated with higher surface area and implementation of other cannulas, but we are convinced that the benefits are for obese patients more important.
Conclusions: Parallelizing the inflow and outflow cannulas is a promising way to lower risk of stroke complications during ECMO support of obese patients.

P216 (EI0151)
THEORETICAL PREDICTION OF ALVEOLAR GAS FRACTIONS USING VARIABLE ARTIFICIAL DEAD SPACE VOLUME
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Objectives: The Dead space volume (V_D) is the volume of respiratory gas which does not cooperate in the pulmonary gas exchange; such volume tends to rise in the case of patients undergoing anesthetic conditions, e.g. tracheal intubation, mechanical ventilation, and also for therapeutic reasons for improvement of lung function, thus causing changes within the alveolar gas content and in turn affecting blood-gas content. Here, we illustrate a theoretical model (based on experimental validation) enabling prediction of alveolar gas composition according to overall extended V_D.
Methods: Numerical balancing is applied according to a 3-step method taking into consideration respiratory parameters. The 1st step is air entering to the alveolar level. The 2nd step is by applying the alveolar gas transport equations and the 3rd step is balancing at atmospheric level, equivalent to alveolar gas volume (O_2 or CO_2) mixed with dead space gas volume. The respiratory volumetric parameters are measured (Wright Respirometer) and the partial gas data as well as gas fractions are monitored (Radiometer, Draeger).
Results: According to computational balancing by maintaining a Tidal Volume up to 750mL and a breathing frequency of 14breaths.min^{-1}, an increase of 450mL V_D will result an alveolar CO_2 partial pressure P_{ACO2}=42.25mmHg. Such results showed accordance with arterial gas data reported in the literature by maintaining the same respiratory conditions which is an arterial CO_2 partial pressure P_{ACO2}=42 mmHg.
Conclusions: It is demonstrated that the theoretical approach is in accordance to the laboratory measured data and can be used for practical applications such as optimizing of e.g. functional dead space as respiratory tubes for therapeutic but also for sport training purposes.

P217 (EI0152)
CFD MODELLING OF ROTARY BLOOD PUMP
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Objectives: The aim of this paper was to calculate hydrodynamical stresses in rotary blood pumps.
Methods: We have used CFD method for calculations of hydrodynamical stresses and energy dissipation distribution in little different geometry of rotary blood pumps.
Results: As a results we have obtained color maps of pressure, velocity, stresses as well as energy dissipation. On the basis of this maps, we are able to predict which pump will cause less hemolysis.
Conclusions: Analyzing the obtained results we can state that the least hemolysis causes pump with canal propeler. We have noticed the least energy dissipation as well as area of large energy dissipation was the smallest for such a pump.

P218 (EI0250)
MODEL OF THE ARTERIAL SYSTEM WITH INTEGRATED MYOGENIC, METABOLIC AND ENDOTHELIAL PERIPHERAL CONTROLS FOR THE COMPARISON BETWEEN CONTINUOUS AND PULSATILE PERFUSION DURING CARDIAC SURGERY
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Objectives: Models used to simulate non-physiological cardiovascular conditions are usually passive models, but the effects of peripheral local regulation are not negligible when studying alterations in vascular system physiology that imply oscillations in microvessel diameter. A large literature is available about myogenic and metabolic controls, while only one model allows evaluating separately the effects of endothelial regulation. This work aims at developing a single controlled model useful to evaluate the variations in micro-vessel diameter during cardiac surgery when continuous or pulsatile perfusions are used.
Methods: Control mechanisms have been integrated and interfaced with a lumped-parameter model of the systemic circulation, consisting of large artery segments and peripheral networks. Myogenic and endothelial controls act on arterioles, metabolic control acts at venules level. Particular attention was paid to the integration of the simultaneous action of myogenic and endothelial control on arterioles. The model was developed using Visual C^{++} language and LabVIEW™ 7.1 software was used for the graphical interface. The effects of either single control activation or multiple controls simultaneous action were evaluated under physiological and pathological conditions. The model was applied to study the variation in peripheral vascular diameters under continuous or pulsatile perfusion in order to explain the clinical evidence of poor organ perfusion under continuous or pulsatile cardiopulmonary bypass.
Results: The developed lumped parameter controlled model allows simulating both physiological and pathological conditions as well as cardio-surgical procedures. According to the experimental evidence, the main results obtained from the model revealed a widespread vascular constriction under continuous perfusion with respect to pulsatile.
Conclusions: The developed model appears flexible and reliable allowing highlighting the effects of local control mechanisms that play a leading role when pathologies or cardio-surgical procedures were considered.

P219 (EI0113)
VIRTUAL RESPIRATORY SYSTEM IN THE EVALUATION OF CLINICALLY APPLICABLE MODELS
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Objectives: Models that can be directly applicable in clinics are usually simple because of difficulties in fitting a complex model to data obtained for individual patients. Therefore, the simplest model of the respiratory system (RS), the RC-model with the resistance (R) and compliance (C) represented by two single numbers, is most frequently used in both diagnosis and treatment. Since, however, the true RS is much more complex and sophisticated than simple models (e.g. nonlinear and/or changeable properties of RS), it should be tested each time whether a model is appropriate for a particular clinical application or not. As the use of real human beings in experiments is limited by serious legal and ethical restrictions, virtual patients (an aggregate of comprehensive models) can be utilized for such tests.
Methods: The virtual cardio-pulmonary system elaborated previously in the Institute of Biocybernetics has been used in some of such tests.
Results: The system was used to determine when the RC-model could be helpful in spirometry interpretation. The most fundamental questions are: 1) which parameters of the real RS influencing spirometry can correspond to the model parameters R and C, 2) how to examine an individual patient to make this correspondence actual. Virtual examinations showed that physically measured resistance and compliance depend on many measurement conditions, and thus - depending on these conditions - the same patient may be characterized by the RC-model having various values of R and C. Certainly, such a result cannot have clinical meaning, at least in spirometry interpretation. Virtual experiments showed that, for example, measurement should be performed in such a way to connect R with resistance of airways after maximal inspiration.
Conclusion: Virtual patients may be used instead of real patients in the initial evaluation of simpler but clinically applicable models and measurement methods.

P220 (EI0032)
MULTISCALE MODELING OF THE HUMAN LIVER BLOOD CIRCULATION: FROM THE HEPATIC MACROVASCULATURE TOWARDS THE MICROVASCULATURE
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Objectives: Computational models of an organ vasculature may provide insight

into organ hemodynamics, perfusion and (dys)function (e.g. transplant research). Previously, we developed an electrical analog model of the human hepatic circulation, based on measured anatomical data of the macrocirculation. However, this model requires refinement, especially at the microcirculatory level.
Methods: Vascular corrosion casts of two human livers (discarded for transplantation) were obtained by simultaneous injections of Batson's™#17 through the hepatic artery (HA) and portal vein (PV). We previously reported data obtained from an in globo micro-CT scan (resolution ±110µm) of one of the liver casts. As this only allowed assessing 5-6 generations, we dissected a lobe and a small sample (±0.134mm³) from the cast and scanned these at higher resolutions (±71µm and ±2.6µm, respectively). Image processing was performed to obtain 3D reconstructions up to the terminal microcirculatory level. These reconstructions enabled measurements of the branching topology (diameters, radii, lengths), and assessment of the microvascular porosity (void volume divided by total volume).
Results: The dissected lobe dataset resulted in the visualization of higher order blood vessel generations [13 for the HA and PV, 10 for the hepatic veins (HV)]. Exponential relations [$y=a\exp(bN)$ with N=generation number] were determined based on data from the 1st to 13th/10th generation, relating generation number to radius, length and number of vessels. For the HA/PV/HV radii [mm], *a* was 4.22/10.26/13.52, while *b* was -0.31/-0.37/-0.48, respectively. The smallest sample showed a very complex network of interconnected and intertwined sinusoids (diameters of ±5-10µm), and the porosity was estimated at 0.148±0.007.
Conclusions: We gathered anatomical and morphological data on the hepatic macro- and microcirculation, forming the basis of a multiscale model of the liver blood flow and perfusion. The application of this method could also be extended to other organs, such as kidneys.

Natural-based polymeric biomaterials and composites for regenerative medicine

P221 (EI0369)
NOVEL GELLAN GUM HYDROGELS FOR TISSUE ENGINEERING OF INTERVERTEBRAL DISC
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Objectives: Centrally situated in the intervertebral disc (IVD) structure, the nucleus pulposus (NP) is a gel-like tissue containing proteoglycan (PG) such as versican, biglycan, decorin, fibromodulin, luminican and especially aggrecan. NP has an important structural role in the IVD, and upon damage it possesses a limited self-repair capacity. Therefore, the main purpose of this work was to develop novel gellan gum-based hydrogel (GG) formulations consisting of GG MPs dispersed in a GG matrix for finding application as an NP substitute.
Methods: Several GG MP/hydrogels discs formulations were prepared by means of mixing high and low acyl GG at different ratio, namely 75%:25% (v/v); 50%:50% (v/v), 25%:75% (v/v); HAGG 0.75% and LAGG 2%, respectively. The GG MP/hydrogel discs formulations were investigated by dynamic mechanical analysis (DMA), swelling behavior and degradation rate. The possible cytotoxicity of MP/hydrogel discs leachables was screened *in vitro* by means of using a rat lung fibroblast-like cell (L929 cells) line. In order to qualitatively investigate the encapsulation efficacy of L929 cells into the GG MP/hydrogel discs a Live/Dead cell viability assay was also carried out.
Results and Discussion: The developed GG MPs/hydrogel discs were physico-chemically characterized by FTIR and ^{1}H-NMR, and GG MPs size was measured by a stereomicroscope by means of staining the MPs with Toluidine Blue-O. From DMA analysis, we observed that the optimal outcome to reinforce GG matrices may be in the range of 50-500 mg/mL of incorporated MPs. The cell culture studies demonstrated that MP/hydrogels discs are non-cytotoxic over L929 cells. Complementarily, it was also demonstrated that L929 cells can be successfully encapsulated in the GG MP of different formulation and that were viable after 72 hours of culturing.
Conclusions: The developed GG MPs/hydrogel discs are promising hydrogels for being used in IVD tissue engineering applications.

P222 (EI0323)
SUBCRITICAL SINTERING OF AN ALIPHATIC POLYESTER ENRICHED WITH ULVAN CAPSULES LOADED WITH A BIOACTIVE AGENT
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Objectives: A marine derived polysaccharide, ulvan, extracted from green algae, was combined with Poly-DL-Lactic acid (PDLLA) in order to produce a novel scaffold targeted for Tissue Engineering (TE) applications. The combination of this natural origin polysaccharide with PDLLA aimed at the improvement of the scaffold performance and physical integrity while broadening drug release capabilities.
Methods: 3D scaffolds of PDLLA loaded with ulvan beads were prepared by subcritical fluid sintering with carbon dioxide at 40ºC and 50 bar. The prepared matrices were further characterized by several techniques as to assess their feasibility as scaffolds for bone regeneration, namely mechanical compression testing, water uptake and degradation studies, morphological characterization by micro-computed tomography (µ–CT) and scanning electron microscopy (SEM) and standard biocompatibility tests (ISO/EN 10993). Effectiveness of ulvan particle loading with dexamethasone within the PDLLA matrix was monitored in terms of release profile of the compound by UV spectroscopy.
Results and Discussion: PDLLA/ulvan 3D structures exhibited a compressive modulus of 8.3±1.51 MPa, a maximum water uptake of 8% and weight loss of 5%, after 21 days. In terms of morphometric properties, these scaffolds presented a porosity of 75.7±1.24%, pore interconnectivity 48.7±8.49% and a mean pore diameter of 268.5±12.02µm. The release of dexamethasone from the ulvan particles demonstrate that the systems designed can be successfully used for *in situ* delivery of bioactive agents.
Conclusions: The obtained results demonstrated the feasibility of processing PDLLA/ulvan 3D scaffolds for potential TE scaffolds. These structures presented appropriate mechanical and morphological characteristics to be used as a scaffold for cancellous bone tissue engineering. They also contributed to the establishment of ulvan as an emerging biomaterial candidate for TE applications.

P223 (EI0394)
***IN VIVO* STUDY ON THE ANGIOGENIC POTENTIAL OF GELLAN GUM-BASED HYDROGELS FOR APPLICATION IN NUCLEUS PULPOSUS REGENERATION**
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Objectives: Tissue engineering of nucleus pulposus (NP) offers a promising alternative strategy to current ineffective clinical approaches for treating intervertebral disc degeneration. Gellan gum-based hydrogels (ionic- and photo-crosslinked methacrylated gellan gum) have been recently proposed as potential candidates for NP regeneration. An important feature of these hydrogels will be their capacity to control blood vessel growth, since the NP is naturally avascular. Our aim was to investigate *in vivo* the angiogenic/antiangiogenic potential of the developed hydrogels, using an optimized adaptation of the chorioallantoic membrane (CAM) assay.
Methods: Sterile hydrogel discs (n=10) made of gellan gum, ionic- and photo-crosslinked methacrylated gellan gum were placed on the CAM at day 10 of embryonic development. Positive (filter paper or gelatin sponge with VEGF) and negative (filter paper or gelatin sponge) controls were also tested. The assay proceeded until day 14 or 18 of embryonic development and images were acquired *in ovo* and *ex ovo* using a stereomicroscope by the end of the assay. The images obtained were image-processed using the ImageJ program for facilitating the counting, which was performed by three independent observers.
Results: The evaluation of the angiogenic response was performed by analysing the convergence of the blood vessels toward the implanted discs. Some degree of variability was found between replicates and inflammation occurred frequently, which hindered the analysis of the formation of new blood vessels. The reduction of the assay duration (from 18 to 14 days) resulted in a decrease of inflammation/contamination. All the materials were partially adsorbed during the assay. However, the controls did not present a regular response and the gelatin sponge was often completely adsorbed.

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Conclusions: The *in ovo* quantification method was more complex as compared to *ex ovo*. The results indicate that no differences exist between the hydrogels tested in what concerns to their angiogenic potential.

P224 (EI0300)

A GREEN APPROACH TO PROCESS SEMI-CRYSTALLINE NATURAL-BASED POLYMERS FOR TISSUE ENGINEERING APPLICATIONS

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Objectives: Following the green chemistry philosophy, this work aims at designing and developing new 3D architectures of natural-based polymers, combining ionic liquids (ILs) and supercritical fluid technology, with relevant applications in tissue engineering and regenerative medicine. With this purpose, SPCL, a polymeric blend of starch, which is one of the most abundantly occurring natural polymers and poly(ε-caprolactone), a synthetic biodegradable polymer, was processed by supercritical fluid foaming, at different operating conditions. The use of this technique for processing natural-based polymers has been limited due to the fact that they are normally semi-crystalline polymers. This can be overcome by the use of ILs, which have recently been proposed as plasticizing agents of starch.
Methods: The IL tested in this work was 1-butyl-3-methylimidazolium acetate and its plasticizing effect was demonstrated by the mechanical tests conducted. The production of porous and interconnected structures was carried out, hereafter, using CO2 as foaming agent. The effect of different operating variables, such as pressure, temperature and contact time on the porosity, interconnectivity and pore size distribution of the matrixes was evaluated and the morphology was analyzed by micro-computed tomography.
Results and Discussion: The results obtained suggest that the induction of porosity within the constructs depends largely on the diffusion of CO2 in the matrix, which explains the higher porosity of the samples processed at higher pressures and larger contact times. Moreover, the presence of IL has been shown to have a key role in the success of the supercritical foaming process, and consequently on the preparation of porous and interconnected scaffolds.
Conclusions: To our knowledge it is the first time that this approach has been reported. The findings described in this work can be extended and adapted to other raw materials, which largely broads the spectrum of natural-based polymers that may be processed into 3D porous matrixes.

P225 (EI0299)

PLLA-PEG CRYOSTRUCTURED SCAFFOLDS REINFORCED WITH BIODEGRADABLE FIBERS

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Objectives: Poly(l-lactide) (PLLA), poly(glycolide) (PGA), poly(ethylene glycol) (PEG) and/or copolymers of these have extensively been used in medicine for applications. VICRYL® is a commercially available suture produced from a copolymer of glycolide/lactide (90/10 mol/mol). In this study, Vicryl® fibers were used for increasing mechanical properties of PLLA-PEG cryostructures.
Methods: PLLA-PEG block copolymer was synthesized by ring opening polymerization of L-lactide dimer and PEG (6000 Da). Wet spun fibers were obtained from chitosan solution in acetic acid. Commercially available Vicryl® was used as a source of Poly(lactide-co-glycolide) (PLGA) fibers. PLGA fibers with varying length (1, 2 and 4mm) and matrix/fiber ratios (2:1, 3:1 and 4:1) were dispersed in % 5 (w/v) solutions of PLLA-PEG in 1,4 Dioxane. Porous scaffolds were prepared with these solutions under cryotropic conditions (-12°C).
Results: FTIR, and NMR spectra confirmed the chemical structure of PLLA–PEG copolymer. Cryostructures made from this copolymer had interconnected macropores, which were obtained. They exhibited remarkable properties, including high flexibility and rapid size change to external forces, and also "swellability" in aqueous media. Vicryl fibers (both the amount and the fiber aspect ratio) increased the mechanical strength of the PLLA-PEG cryostructures quite significantly.
Conclusions: PLLA-PEG cryostructures reinforced with vicryl fibers were concluded to be a good candidate that can be used in tissue engineering applications for both hard and soft tissue regeneration.

P226 (EI0292)

VALORIZATION OF CHITOSAN FROM SQUID PENS AND FURTHER USE ON THE DEVELOPMENT OF SCAFFOLDS FOR BIOMEDICAL APPLICATIONS

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Objectives: The aim of the present work is the valorization of squid pens through the production of chitosan that can be used for the development of biomedical applications. The present work is focused on β-chitin extraction from squid pens of the species *Dosidicus gigas* and its further conversion into chitosan. The biomedical potential of the isolated squid chitosan was assessed by processing this polymer as scaffolds for tissue engineering strategies.
Methods: Alkali solution was used to deproteinized squid pens and thus isolate β-chitin, which was further converted into chitosan through a deacetylation reaction. The chitosan scaffolds were developed using a freeze-drying process, from 3% and 4% chitosan solutions in acetic acid and freezing at temperatures of -80°C and -196°C. Chitosan scaffolds were neutralized using two different methods: M1 – NaHO solution; and M2 – ethanol/water and NaHO solution. Morphology, Mechanical properties, degradation, cytotoxicity (L929 cells) and cellular adhesion (ATDC5 Chondrocytes like cells) of squid chitosan scaffolds were assessed and compared with the properties of scaffolds produced with commercial chitosan.
Results: The morphology of scaffolds revealed a lamellar structure for all produced scaffolds, independent of the origin and concentration of chitosan. The treatment with sodium hydroxide and ethanol caused the formation of larger pores and loose of some lamellar features. Different freezing temperatures gave different pore morphology and the lower temperature a smaller pore size. The *in vitro* cell culture and cell adhesion studies showed that all chitosan scaffolds exhibited a non-cytotoxic effect over the mouse fibroblast-like cell line, L929 cells.
Conclusions: The chitosan produced from the endoskeletons of giant squid *Dosidicus Gigas* has proven to be a valuable alternative to the commercial one when considering its use as biomaterial for different biomedical applications.

P227 (EI0203)

ENCAPSULATION OF HUMAN MESENCHYMAL STEM CELLS VIA PROTEIN CROSS-LINKING FOR INTERVERTEBRAL DISC REGENERATION

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Objectives: Low back pain is a common disease in modern society. One major reason is the degeneration of the nucleus pulposus (NP, part of the intervertebral disc). The regeneration of nucleus pulposus via cell therapy requires a biocompatible matrix, which facilitates the differentiation of hMSCs (human mesenchymal stem cells) to nucleus pulposus cells. The enzyme transglutaminase was found to be suitable to generate a gelatine matrix by cross-linking and could be utilized to immobilize hMSCs. Further natural ECM of the NP should be used for crosslinking and immobilization.
Methods: The immobilized cells were cultivated for 21 days in media, containing growth and differentiation factors. Cells were seeded at a density of $4x10^6$ cells per cm^3, equal to the density in the NP. Viability of the cells in the gelatine matrix was proofed by using a tetrazolium salt (WST-1). At certain dates RNA isolation was done following the phenol/guanidine isothiocyanate protocol. RNA was transformed into cDNA followed by a RT-PCR with a gel electrophoresis afterwards. Different primers were used to analyze the successful differentiation of hMSC-TERT into nucleus pulposus cells. In further analysis, NP extract isolated from pigs, has been added to the gelatine matrix.
Results: The viability of the immobilized cells has been on a constant value over the differentiation period of 21 days. Thus, the survival of hMSC-TERT in gelatine is proofed. The differentiation status of hMSC-TERT in the two different matrices (gelatine, NP extract) could be analyzed.
Conclusions: Data concerning the differentiation of hMSC-TERT in nucleus pulposus cells in a gelatin matrix and differences between the two matrices will be presented.

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Stents and Vascular Implants

P228 (EI0339)

VALIDATION OF A TEST SETUP FOR HEMOCOMPATIBILITY TESTING OF SMALL CARDIOVASCULAR IMPLANTS

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Objectives: Many of the parameters that have substantial influence on blood damage in *in vitro* hemocompatibility testing frequently remain undefined, leading to poor reproducibility. Taking into account previous research and prevailing lack of comparability, our goal is to develop a simple and reliable dynamic *in-vitro* test setup for testing small cardiovascular implants, such as stents.
Methods: To keep dilution effects low, circulating blood volume was restricted to a minimum. As previous experiments have shown that roller pumps are not suitable for hemocompatibility-testing with small blood volumes, we used the well-known Chandler-loop mounted on a standard laboratory pump drive. All experiments were conducted with bare metal stents and heparinized porcine blood at 37°C. Three stents in a row were placed in polyurethane tuberings with an inner diameter of 2.4mm. Hematocrit, hemolysis and platelet count were determined at 0, 1 and 4 hours. The tuberings then were rinsed with 0.5% Octoxinol 9 (Triton X) and reused to rerun experiments four times.
Results: After 1 hour platelet count in tube rings containing stents had dropped by an average of 17% compared to reference tuberings while hematocrit and hemolysis remained unchanged. Similar results were obtained after 4 hours. The measured effect decreased after each rinsing procedure, but only for the tuberings containing stents. This indicates that stent surfaces underwent a change while in contact with blood that cannot be completely reversed by rinsing with Triton X.
Conclusions: Unlike roller pumps, the Chandler-loop causes little initial damage to blood. The effect of bare metal stents on platelet count was clearly detectable after 1 hour, whereas rinsing diminished detectable differences. Experiments with surface-treated stents are currently in progress. The setup will also be used to test electrospun matrix components for vascular prostheses for hemocompatibility within a DFG-funded collaborative research centre.

P229 (EI0402)

MECHANICAL CHARACTERIZATION OF VERY SMALL BLOOD VESSELS AND VASCULAR GRAFTS

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Objectives: The determination of biomechanical properties of small diameter vascular grafts and very small vessels is an important task. Apart from optimal adaptation of graft compliance to natural vessels, also biomechanical investigations are essential in frequently used animal models such as from rats and mice. In this work a method was established for testing mechanical behavior of very small vascular prostheses and natural vessels of less than 1mm diameter by the use of a modified tensile testing machine.
Methods: The mechanical behavior of electrospun vascular grafts and of murine thoracic aortae was measured. For this purpose a BOSE ElectroForce testbench system with a 200 N Linear motor (Bose Corp. MN, USA) was modified with a cantilever and probe fixation for ring-shaped specimens, which allows the application and measurement even of very small forces. Rings of 2mm width were cut out of the aorta descendens. In the testing system, the rings were placed between two 0.2mm pins and loaded in circumferential direction until rupture. Force–elongation curves were recorded. Maximum force, elongation and compliance in the physiological range were investigated.
Results: For mice after 19 weeks high-fat diet (B6.129P2-ApoE/J, age: 254±9 days), the test showed a maximum force of 0.41±0.12 N. The strain at maximum force was 84.3± 5.5%. Maximum forces and strains varied within the different zones of the aorta. The compliance at 100mmHg was calculated out of the measured data, using Laplace's law and was 36±6.3% / 100mmHg.
Conclusions: The established method allows a reproducible and sensible measurement of mechanical properties in very small ring-shaped specimen of arteries and vascular prostheses.

P230 (EI0289)

DEVELOPMENT OF A SMALL-CALIBER AUTOLOGOUS VASCULAR GRAFT "BIOTUBE"; FOUR-YEAR ANIMAL IMPLANTATION

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Objectives: Since ESAO 2005, we have reported that *in vivo* tissue-engineered autologous tubular tissues "BIOTUBEs" are useful as small-caliber vascular grafts in rat, rabbit and beagle dog models. Their autologous connective tissue walls (thickness: ca. 0.1mm) exhibited rapid regeneration of hierarchical vascular wall structure. In this study, we report long-term implantation of Biotubes in rabbit and beagle dog models.
Methods: Silicon rod molds (diameter: 2~5mm, length: 20~50mm) were placed into dorsal subcutaneous pouches of rabbits or beagle dogs. After 1 month, BIOTUBEs formed around the molds were auto-implanted to the respective animals.
Results: In both kinds of animals, after removing the molds from the implants harvested with around connective tissue, BIOTUBEs mostly consisting of coarse autologous collagen fibers and fibroblasts were obtained. BIOTUBEs showed burst strength more than 1500mmHg. *Rabbit*: Total patency rate of 3 months is 81.8% (n=11). Longest follow-up is 2 years without any degenerative changes. Little thrombus was formed on the luminal surfaces completely covered with endothelial cells with parallel orientation to the direction of blood flow. With time, hierarchical arterial structures were reconstructed in the recipient body under hemodynamic conditions, including circumferentially oriented smooth muscle cells, collagen fibers bundles and elastin fibers. *Beagle dog*: Rapid tissue regeneration was observed in spite of the animal species. Angiography at 2 years (n=1) and ultrasound tomography at 3.5 years (n=1) and 4 years (n=1) showed the graft patency, neither stenotic changes at anastomosis nor degenerative dilatation.
Conclusions: Autologous BIOTUBEs with no synthetic support materials withstood systemic blood pressure and exhibited excellent performances as small caliber vascular prostheses for up to 4 years. Further studies with numbers are ongoing for the aim of future clinical applications.

P231 (EI0164)

THE CORE LABORATORIES FOR THE INVESTIGATION OF CARDIOVASCULAR EXPLANT MATERIALS HEART VALVE PROSTHESES AND STENTS STUDY

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Objectives: The Core Laboratories for the investigation of failed cardiovascular implant materials, initiated by to UE grant COST Action 537, have been organized by the Foundation for Cardiac Surgery Development in Poland. *Objectives:* For FCSD, a research centre working on heart prostheses, biomaterials and new surgery tools the goal is the improvement of medical devices in clinical practice from the analysis of implanted prostheses. The Physician, supported by a scientific multidisciplinary team can recognize the reason of the surgical intervention and exchange the prostheses.
Methods: The samples were harvested during routine explantation or substitution by other prostheses or during post-mortem examination. The combined use of different methodologies for the explants analysis provided complementary information on both the material reaction to the biological environment and the host response to the implant. Our database consisted of the scanty clinical information, initial surface studies and histological studies. Following gross inspection and photographing, the devices were analyzed by optical (OM) and electron microscopy (SEM), Energy Dispersive X-ray Spectroscopy (EDX), Attenuated Total Reflection Fourier Transform Infrared Spectroscopy (ATR-FTIR). Radiographic examination, microbiological analysis, biological evaluation of adsorbed proteins, and histological analysis were conducted on the materials and peri-implant tissues. Biological analysis included Electrophoresis and Western Blot for the evaluation of the proteins adsorbed on the different device components, and ELISA tests for specific protein quantification.
Results: About 150 valves and 40 vessels prostheses and about 30 vascular stents have been stored and banks of explanted prostheses have been set up. The several kinds of valves prostheses damage have been modeled and tested. The few of our last unique results is the Edwards SAPIEN Transcatheter Heart Valve and heart coronary artery stent study.

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Conclusions: The report of achieving results and consideration about possibility for using it for advisory system knowledge bank have been prepared.

P232 (EI0143)

DEVELOPMENT OF A POLYURETHANE-VALVED CONDUIT WITH VALSALVA SINUSES FOR THE RECONSTRUCTION OF THE RIGHT VENTRICULAR OUTFLOW TRACT (RVOT)

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Objectives: Around 20% of congenital heart defects require a surgical reconstruction of the right ventricular outflow tract (RVOT), often performed using valved conduits. Several studies have already proven the importance of Valsalva sinuses in such prostheses. Currently existing prostheses require permanent coagulation (mechanical prosthesis), have limited durability (xeno/homografts) and availability in small diameters. Thus, the aim of this work is to design and manufacture a complete prosthetic polymeric valved conduit with Valsalva sinuses.
Methods: A two-part core which ensures sinus formation and valve integration into the conduit was manufactured. The conduit was then produced by polyurethane atomization (spraying), allowing the production of a fine-fibrous structure with a special micro-porous surface, which can favour a neo-intimal layer formation. The micro-porous structure was observed and studied under microscopy. The prostheses mechanical and structural properties - tensile strength, compliance, burst strength, permeability and suture retention - as well as the valve functionality were subsequently tested *in vitro*.
Results: Laboratory samples with a 22mm internal diameter were produced for *in vivo* experiments in calves. Structure variations were applied through process parameters changes. Valve opening and closing was investigated with high-speed video and functionality was assessed for pressure differences of 20, 40 and 80mmHg. The conduits showed sufficient compliance to propagate the pressure pulse and to withstand pressure peaks. The prostheses were impermeable up to 100mmHg pressure. The tensile, suture retention and burst strength were satisfactory.
Conclusions: The production of valved conduits is possible using polymer atomization. *In vitro* tests showed satisfactory results for mechanical properties and functionality. These results encourage the optimization and conduction of further research in this field. A chronic implantation of the conduits in calves is planned and results are expected August 2011. Further *in vivo* experiments and *in vitro* fatigue testing will be carried out to assess the conduits durability.

P233 (EI0144)

IMPROVEMENT OF STATIC PERFORMANCES OF BIOMORPHIC POLYMERIC HEART VALVE PROSTHESES BY TAILORING THE MATERIAL ORIENTATION

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Objectives: Some styrenic block copolymers (SBC) having cylindrical morphology allow tailoring the microstructure to achieve anisotropic mechanical behaviour similar to natural tissues. The aim of this study is to investigate numerically the effects of material structure orientation on the performance of biomorphic symmetric tri-leaflet heart valve prostheses (HPV).
Methods: Two oriented SBC (Kraton 19%wt-polystyrene from Kraton Polymers and V4111 19%wt-polystyrene from Dexco Corp.) were mechanically characterized. A hyperelastic anisotropic constitutive model was implemented into a commercial finite element code. A parametric CAD of a biomorphic symmetric HVP (size 22x12x0.35mm) in closed configuration was developed. Exploiting symmetry, a single leaflet was discretised into linear hexahedral elements. Uniform static pressure (180mmHg=24kPa) was applied. The code was equipped with an iterative algorithm for the reorientation of the fibres along the local maximum principal stress (MPS) direction, seeking for optimality conditions. The numerical data were analyzed in terms of stress distribution, coaptation area (CA), and vertical leaflet slipping (VS), used as indexes of the HVP global performance.
Results and Discussion: Kraton leaflets present a more uniform stress state (MPS<0.4MPa for 60% of the elements). The maximum decrement of VS for this polymer is 16.4% with respect to the baseline calculation while V4111 displayed no significant changes. For both materials, maximum CA for optimised leaflets was 1% larger than the corresponding baseline, confirming that the coaptation mechanism is mainly driven by the flexural stiffness of the structure rather than by the tensile stiffness of the material.
Conclusions: V4111 leaflet showed no significant enhancement of the mechanical and structural performances when optimized. Kraton leaflets showed a reduction of the VS at constant CA, denoting stabilisation of the closure of the valve. The study demonstrates that optimization of the microstructural orientation of the material can enhance the performance of a polymeric HVP.

P234 (EI0226)

FLUID DYNAMICS CHARACTERIZATION OF REGURGITANT FLOW IN MECHANICAL HEART VALVE

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Objectives: The regurgitant flow in mechanical heart valves has been frequently associated to blood trauma (hemolysis, platelet activation). In these devices, a certain amount of regurgitant flow is a desirable option, since it provides washout of the hinges, where blood particles, if trapped in low-velocity regions, could initiate aggregation phenomena and ultimately lead to thrombosis of the valve. Thus, regurgitation in mechanical valves is meant to maintain the valve surfaces clean of blood deposits; on the other hand, regurgitant-flow jets can have detrimental effects on blood, due to flow-related stresses.
Methods: In order to characterize valve regurgitation, a physical model of the regurgitant flow, previously built at the ISS' premises, was used. The prosthetic valve under test was seated coaxially with a 12-face prism, enabling easy optical access. This model was inserted in a closed flow loop, in which the regurgitant steady flow is driven by 80mmHg transvalvular pressure, preset by positioning a reservoir at a suitable position above the valve plane. A Particle Image Velocimetry investigation was carried out on currently marketed bi-leaflet valves, by measuring the 2D flow field at several planes encompassing the plane of the two distal hinges. Mean and fluctuating velocities were calculated over 1000 instantaneous measurements. Maximum turbulence shear stresses (TSSmax) were also calculated, by means of Principal Stress Analysis.
Results: The study enabled to reconstruct tomographically the jets exiting the valve. This information can be used as a reference standard for improving the assessment of valve function during echocardiography. Very low TSSmax values (not exceeding 12Pa) were found, confirming the limited impact on blood of the tested valve.
Conclusions: The experimental set-up provided an accurate characterization of the leakage phase, which has gathered increasing attention in the recent past, due to its potential contribution to thrombogenicity of cardiovascular implants.

P235 (EI0225)

COMPUTER-AIDED ANATOMICAL FITTING AS A METHOD TO IMPROVE THE DESIGN OF CARDIOVASCULAR IMPLANTS

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Objectives: Good integration of cardiovascular implants with the surrounding anatomy is an important implantation criterion. In this study the method of virtual fitting to optimize implant design was evaluated and compared to cadaver studies. The design of a Total Artificial Heart (TAH) was reviewed and optimized with regards to inlet and outlet orientation.
Methods: Computer Tomography (CT) scans of 13 female and 10 male patients (ages 45-91) with cardiovascular disease were reconstructed into 3-dimensional geometries using a grey scale threshold and different segmentation algorithms as well as manual editing. Absolute location of all valves was identified and key parameters were calculated including valve diameter, orientation in global 3D space and relative distance to key markers in the thoracic cavity. The valve parameters were averaged to determine optimal inlet and outlet positioning for overall fit. The new CAD TAH geometry was superimposed on CT geometries to verify volume fit within the anatomical space. Using models manufactured by rapid prototyping the new configuration was verified in seven cadaver studies. Additionally three of the cadaver anatomies were generated into computational geometries using a 3D-coordinate measuring system and compared to the CT data.
Results: CT valve parameters varied between 13% and 17% and were higher in male than female patients (M:15-23%, F:10-14%). The valve parameters measured during the cadaver studies were similar to those acquired *in silico*, however the variation was slightly higher (17-34%). The inlet and outlet configurations

of the TAH chambers could be matched to the physiological values.
Conclusions: Digital Fitting of cardiovascular implants is a powerful tool in the development of new devices. This method can not only be used for preliminary design optimization of medical devices, thus reducing the number of cadaver studies, but also provides a method to examine fitting in anatomical environments unaltered by sternotomy.

P236 (EI0129)
VASCULAR PROSTHESES MANUFACTURED BY SPRAY-ATOMIZATION OF POLYMERS
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Objectives: The use of porous materials for vascular prostheses can give some advantages over continuous surfaces. Porous surfaces encourage the formation of a thin neo-intimal layer onto the material, as has been shown by Karapinar et al. Matching the mechanical properties of the graft with physiological values also benefits the acceptance of the graft in the body. It is therefore highly desirable to produce porous materials from biocompatible polymers. Our goal is to develop a manufacturing process for vascular grafts, which produces thin fibers from a polymer solution, and has a higher throughput than electrospinning.
Methods: A pneumatic spray gun is used to atomize a polymer-solution and to form small droplets, which then dry and deform to fibers during their flight to the target. The nearly dry fibers then form a non-woven textile on the target. The target can rotate and the spray gun can move in two axes. Factors like atomization pressure, material flow, solvent, concentration and polymer used can also be varied. Sprayed samples are inspected by microscope and subjected to tensile tests. Sterilization tests have been conducted with gamma, ETO, plasma and steam sterilization.
Results: It is possible to spray fibers with a diameter of 1µm. The permeability of the textiles can be influenced by changing spraying parameters. Different polymers (PCU and PLA) have been successfully tested for this manufacturing process. The PLA can be used as a scaffold for tissue engineering, whereas the PCU can be used for polymeric graft prostheses. The PCU textiles showed good mechanical properties and a linear stress-strain behavior. The material can be sterilized by gamma, ETO and plasma sterilization.
Conclusions: The spraying process shows promising results for the production of non-woven materials with properties beneficial to their application as grafts and scaffolds.

P237 (EI0210)
DECELLULARIZATION OF BOVINE PERICARDIAL TISSUE FOR TISSUE ENGINEERING APPLICATIONS MAY ALTER ITS BIOMECHANICS AND TISSUE COMPONENTS
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Objectives: We studied the biomechanical behavior and biochemical composition of acellular bovine pericardium (BP) matrix, scoping to a scaffold capable of exhibiting the mechanical properties desired for tissue engineering (TE) applications, especially for the treatment of cardiovascular system deficiencies.
Methods: BP tissue was decellularized according to two different protocols: 1) Treatment based on Triton® X-100 (12h, 4°C); 2) A classic treatment with Trypsin/EDTA at 37°C for 48h. Biomechanical viscoelastic characteristics, high modulus E_H, low modulus E_L and hysteresis ratio (h) were determined from dynamic cyclic tensile stress-strain testing (1 cycle/sec, saline wetted, 37°C). The biochemical determination of tissue components after papain digestion involved chondroitinase digestion for glycosaminoglycans, followed by HPLC analysis and HCl hydrolysis for hydroxyprolin.
Results: Biomechanical characteristics, E_H and E_L found to vary (40 to 50MPa and 0.27 to 0.30MPa, respectively) in fresh untreated tissue, depending on anatomic direction. Decellularization by (1) had no mechanical effect (44.65 to 52.67 and 0.37 to 0.37MPa) while after (2) a significant decrease was found (20.96 to 36.82 and 0.20 to 0.23MPa). Hysteresis (h) ranged (19-26% of the loading energy dissipated), depended on anatomic orientation with no difference. Tissue glycosaminoglycans content was unaffected after treatment (1), while a 22% of chondroitin/dermatan sulfate and 60% of hyaluronan removed after (2).
Conclusions: Decellularization of BP under long duration in 37°C (2) altered its biomechanical behavior, which seemed to be retained under low temperature short duration treatment (1). Decrease of glycosaminoglycans by treatment (2) may reduce biomaterial efficiency as TE scaffold regarding cell emigration, proliferation and function towards regenerative process. Cell-biomaterial interactions study is in progress.

P238 (EI0128)
FUNCTIONING TEST OF VASCULAR SMOOTH MUSCLE CELL CONSTRUCTS
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Objectives: To analyze the efficiency and functionality of smooth muscle-like cell-(SMCl-) constructs, differentiated from adipose tissue derived stromal cells (ADSC) in an innovative test stand.
Methods: ADSCs were differentiated into smooth muscle-like cells in different media (Medium A, B and C). ADSCs and primary SMCs were embedded in collagen-I gel-constructs. After installing the constructs in the testing device, force/time diagrams were recorded. The force generated by the constructs after application of NO, KCl or norepinephrine (NE) was measured.
Results: SMCl-cells differentiated from ADSCs express, like primary SMCs isolated from human carotis, mRNA and proteins required for a functional cytoskeletal apparatus. Collagen-I gel-constructs could be obtained, which remained stable for at least 14 days, comprising cells of both origins. Application of NE or KCl to the culture medium in the test device resulted in the spontaneous contraction of the constructs. Relaxation of the cell-constructs could be observed when the culture medium was supplemented with NO. The extent of the induced constriction or dilation was highest in constructs made of primary, terminally differentiated SMCs. SMCl cells differentiated in medium C exerted higher forces than cells derived from medium A or B.
Conclusions: Patient derived adipose tissue derived stromal cells can differentiate towards smooth muscle-like cells *in vitro*. For the application in a tissue engineered vascular prosthesis the differentiated cells have to prove functionality. With this test stand we are able to analyze the efficacy of contractile smooth muscle differentiation.

P239 (EI0198)
LASER-ASSISTED BIOPRINTING FOR THE GENERATION OF VASCULAR-LIKE STRUCTURES
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Objectives: To generate vascular-like structures by laser-assisted bioprinting (LaBP) for the application in tissue engineered constructs.
Methods: LaBP was applied to seed alternating spots of human endothelial cells (ECFCs) and human adipose tissue-derived stromal cells (ADSCs) in vicinity to each other on a planar recipient plate. The distance between the cell spots was set to 600µm. Both cell types were embedded in a fibrinogen-hyaluronic-acid hydrogel, which was consolidated by a subsequent treatment with thrombin. The printed recipient plate was incubated in endothelial growth medium without the supplementation of VEGF for 10 days. Images were taken every day in order to monitor the development.
Results: First, ADSCs started to grow towards the direction of the nearest spot of ECFCs, which remained quiescent during the first days of incubation. At day 3 ADSCs contacted the ECFCs, which in turn also proliferated and participated in the formation of vascular-like structures. These structures remained stable for the incubation period of 14 days. Furthermore, we could show that LaBP is also capable of printing stacked levels of cell spots in order to create three-dimensional constructs.
Conclusions: LaBP printing of ECFCs and ADSCs in direct vicinity to each other leads to the formation of vascular-like structures. Combined with the possibility of printing in three-dimensions, this technique might be a useful tool for the reproducible generation of tissue constructs with a pre-defined capillary bed.

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P240 (EI0119)

CREATION OF DEVITALIZED VASCULAR PROSTHESES OF SMALL DIAMETER

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Objectives: Existing methods of prosthetic repair in aortal-coronary bypass have some serious shortcomings. There is a deficit of autological prostheses, their isolation traumaticity increases the risk of infectious and inflammatory complications. Allogeneic prostheses are inclined to rejections and thrombosis. Synthetic prostheses of small diameter are not suitable for such a prosthetic repair. The technology of creation of biological prostheses based on xenogeneic vessels has not been effective till now. This study conception is based on the use of two physical factors: low temperatures and irradiation with electrons to devitalize the xenogeneic arteries with preservation of their strength properties.
Methods: The research object was mature porcine intrathoracic arteries, isolated with meeting the bioethical protocols. Procured vessels were subjected to freezing down to -196ºC and irradiation with electrons in the experimental doses. Biomechanical properties were examined with strength- and burst-tests, as well plasticity was estimated. There were performed histological studies of vascular scaffolds, including those after xenogenous transplantation under skin and into system blood flow as vascular prosthesis.
Results: After freezing the vessels vast sites of endothelium desquamation were noted. The following irradiation with electrons causes a complete devitalization of arteries. Structural integrity of connective tissue fibers of vascular wall after irradiation was not impaired. The endurance of arteries in longitudinal direction increased after freezing. When measuring the durability in radial direction a rise in the group of irradiated arteries was found, if compared with native vessels. No acute inflammations were found after implantation of the treated arteries under the skin of rats for all the observation terms. The adequate functioning of the treated vessels was shown during implantation into the system blood flow.
Conclusions: The proposed devitalization method allows the creation of integrally functioning biological vascular prostheses and may be an alternative when selecting the grafts for aortal-coronary bypasses.

P241 (EI0116)

6-MONTH AUTO-IMPLANTATION OF AUTOLOGOUS TISSUE SMALL-CALIBER VASCULAR GRAFTS, "BIOTUBE", TO CAROTID ARTERIES OF BEAGLE DOGS

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Objectives: We have reported that *in vivo* tissue-engineered autologous tubular tissues "BIOTUBEs" could be ideal small-caliber vascular grafts in animal experiments. They are constructed in the recipient bodies safely and economically without any use of special clean facilities. In this study, BIOTUBEs were auto-implanted to the carotid arteries of the beagle dogs, and histological change of BIOTUBEs after implantation was evaluated.
Methods: Biotubes were prepared by placing silicone rods (outer diameter, 5.0mm; length 80mm) used as a mold into dorsal subcutaneous pouches in the beagle dogs for 4 weeks. The obtained BIOTUBEs after nonthrombogenic drug coating were auto-implanted into the carotid arteries of the same beagle dogs by end-to-side anastomosis using 6-0 nylon continuous suture. Graft status was evaluated by digital subtraction angiography. Histological evaluation was performed 3 and 6 months after implantation.
Results: After a 4-week preparation in the subcutaneous tissues, the molds were completely covered with autologous connective tissues (thickness: ca. 0.1mm) mainly consisting of fibroblasts and collagen fibers. During 6-month implantation, neither formation of aneurysms nor rupturing was observed in BIOTUBEs. On the luminal surfaces, endothelial cells were covered partially in the vicinity of the anastomotic region of the grafts at 3 months after implantation. On the other hand, endothelial lining was formed completely at 6 months.
Conclusions: BIOTUBEs could be used as small-caliber vascular prostheses that greatly facilitate the healing process and exhibit excellent biocompatibility in vascular regenerative medicine.

P242 (EI0075)

APPLICATION OF CAD/CAE-TECHNOLOGIES FOR WORKING OUT OF A UNIVERSAL DESIGN OF AN ARTIFICIAL VENTRICLE OF HEART

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Objectives: Working out of a universal design of an artificial ventricle of heart (AVH) for devices of auxiliary blood circulation with an electromechanical and pneumatic drive for application in extracorporeal and implanted systems.
Methods: For the decision of the given problem methods of computer modeling were applied to definition of geometrical parameters of AVH and methods of the certainly-element analysis for a finding of optimum hemodynamic parameters of work of AVH. Geometrical modeling was spent in system Pro/Engineer WF 4. System Ansys was applied to the analysis of a current of fluid in chamber of AVH. Modeling was spent with both a moving, and a stationary membrane with the task of beginning and boundary conditions. The system was put to the test on the test bench for definition of an output and an imaging of a current of fluid in chamber of AVH by application the laser set, which works by cooper steam, model CVL-10 (λ = 510 nm, Russia), and high speed camera, model CCD – SMOS VS – FAST (speed of shooting 5000 fps, Russia).
Results: Hemodynamics key parameters, giving the chance to optimize a design by hemodynamic indicators, are defined.
Conclusions: The model of optimum design of AVH for application in considered designs is received.

P243 (EI0048)

DEVELOPMENT OF CONSTRUCTION TECHNIQUES FOR TISSUE ENGINEERED HEART VALVE SUBSTITUTES

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Objectives: Among the four heart valves, the aortic valve is the one exposed to the most severe flow and pressure conditions, making it more prone to diseases such as stenosis or regurgitation. In order to reduce risks of cardiac insufficiency, diseased valves need to be replaced. Mechanical or tissue valves are often used despite their major disadvantages related to increased risks of immunological response, limited life expectancy and lifetime anticoagulation therapy. On the other hand, tissue engineering offers the possibility to create biological substitutes with the potential to adapt and grow with the surrounding tissues, hence eliminating the need for lifelong medication and risky subsequent surgery.
Methods: A detailed review of the literature allowed us to identify the construction techniques with the most potential. Two methods will be developed. The first one will use a natural scaffold made from decellularized porcine valve from which cells will be removed by chemical, enzymatic and mechanical techniques. Dynamic seeding will follow in a custom made bioreactor designed by our team. In the second construction technique, the tridimensional complex structure of the aortic valve will be reproduced by a manufacturing process using purpose built tools and tissue sheets made with the self-assembly method.
Results: Tissue sheets culture followed by preliminary tests confirmed the feasibility of heart valve construction based on two-dimensional materials. However, further tests will be necessary in order to determine the required thickness of the sheets and then optimizing the developed techniques. Simultaneously, decellularization protocol of the porcine scaffold was established. First tests using this method will be performed in the next months.
Conclusions: A detailed literature review on heart valve replacement technologies, as well as preliminary tests performed by our team, validated that tissue engineering techniques can lead to the development of entirely autologous heart valve substitutes.

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P244 (EI0008)

DEVELOPMENT OF MICROPOROUS SELF-EXPANDING STENT GRAFTS FOR TREATING CEREBRAL ANEURYSMS - DESIGNING MICROPORES TO CONTROL INTIMAL HYPERPLASIA

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Objectives: Treatment of large cerebral aneurysms with a broad neck in the cranio-cervical area is difficult and carries relatively high risks, even with surgical and/or endovascular methods. To this end, we have been developing a high-performance, self-expanding stent graft.
Methods and Results: A commercially available NiT stent (5mm in diameter, 20mm in length) was covered with a thin microporous segmented polyurethane membrane fabricated by the dip-coating method, then micropores were created by the excimer laser ablation technique, and the outer surface was coated with argatroban. Micropores with two patterns were designed. One type had a circular shape (diameter, 100μm; opening ratio, 12.6%), and the other was bale shape (size, 100 x 268μm; opening ratio, 23.6%). Experimentally fabricated side-wall aneurysms of both canine carotid arteries using venous pouches from external jugular veins were placed with the self-expanding stent graft on one side, and with a bare self-expanding stent on the other side. All carotid arteries were patent and free of marked stenosis after 1 month. All aneurysms were occluded by stent grafts, while patent in those treated with bare stents. Histologically, the stent grafts with bale-shaped micropores and a high opening ratio were associated with less intimal hyperplasia (187±98mm) than bare stents (341±146μm) or the stent grafts with circular micropores and a low opening ratio (441±129μm). A pore ratio of 23.6% was found to control intimal growth.
Conclusions: Our self-expanding stent grafts were easily applied to experimental aneurysms, and had achieved complete aneurysm occlusion in beagles at 1 month after implantation. The stent graft with high opening ratio was associated with less intimal hyperplasia than a bare stent or a stent graft with low opening ratio.

Robotics and Instrumentations

P245 (EI0383)

ULTRASOUND LUNG COMETS IN PATIENTS UNDERGOING HEMODIALYSIS

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Objectives: Sonographic B-lines (also called ultrasound lung comets - ULCs) are a sign of extravascular lung water (EVLW). Absent in normal lungs, B-lines rise up as the lung become congested and the interstitium starts to fill with fluid because sound is transmitted through these fluid-filled spaces and reflected between the walls of the congested interstitium. ULCs appear as hyperechogenic bundle with narrow basis spreading from the transducers to the further border of the screen. Aim of this study was to demonstrate the utility and easiness of chest ultrasound to evaluate lung congestion in hemodialysis.
Methods: Ten patients underwent two chest ultrasound examinations: before and after dialysis. We followed a chest ultrasound protocol that counts the number of ULCs visualized in 28 lung zones. For each patient baseline demographics, bioelectrical impedance vector analysis (BIVA), pulse oximetry, thoracic physical examination pre- and post-dialysis, volume of fluid removed, subjective dyspnoea were recorded.
Results: All patients had initial predialysis ULCs >14. After dialysis, seven of ten patients had <14 ULCs. Pre-dialysis ULCs medium score was 34.4±23.5, post-dialysis medium score was 10.7±7.2. Medium volume removed was 2.5L. At the post-dialysis time point, for every 500mL volume removed, there was a decrease of 4.7 ULCs. Nowadays, the methods to evaluate dry weight in patients undergoing dialysis are based on blood pressure, BIVA, presence of oedema and thoracic physical examination. Our data, even if in a little group of patients, support chest ultrasound as an easy, low-cost, useful method for evaluating real-time changes in EVLW in patients undergoing dialysis, being often highly congested.
Conclusions: Chest ultrasound is an easy and low-cost method, useful and less harmful than X ray, to evaluate lung congestion in patients undergoing dialysis.

P246 (EI0171)

SURGERY TELEMANIPULATOR STEERING CONSOLE AS INTEGRATED BIOMEDICAL DATA PROCESSING CENTRE IN MAN-MACHINE INTERFACE OPTIMIZATION

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Objectives: Well-known advantages of minimally invasive surgery (MIS), can be strengthened using tele-manipulation systems for surgery support. Movement scaling, precision, surgeon ergonomy improvement especially during long-term MIS procedures are features of the surgery robotic systems, where surgeon manipulate sitting next to steering console with haptic input device (Master) to control the remote (Slave) manipulator with camera or tool. The goal of the presented work was to optimize this Man-Machine Interface, developed in the project of RobIn Heart tele-surgery system. In assumed approach the Master Console – «RHShell» was treated as an integrated centre of two main data channels: optical channel with 3D visualization and main Master-Slave control system, which maps surgeon hand movement with force feedback pilot study.
Methods: Developed master-slave bilateral control system, transfers scaled and filtered human operator commands from 18 degrees of freedom (DOF) of Master handle to 3 slave arms (2 with surgery tools and 1 for camera channel – total 16 DOFs). It was implemented on the effective Field Programmable Gate Array (FPGA) structures, which allow building their own system on chip with very high data processing, direct on silicon. Visual feedback channel, provided to surgeon in 3D Master console, was innovatively developed in the project using two separated optic channels with empirically chosen distance and angle.
Results: Ergonomy and control effectiveness in real and virtual environment was tested on four different types of Man-Machine interface – space manipulator with spherical structure, foot multifunction unit, medical joystick and manual pilot. Position, velocity and torque mode of control system were implemented and tested during tele-surgery on pig organs (*in vitro*) and two animal experiments (*in vivo*).
Conclusions: Innovations, introduced to impove the Master console, have a crucial meaning for the whole surgery robotic system, which decides of its acceptance in medical world.

P247 (EI0165)

ARTIFICIAL TISSUE MECHANICAL MODEL AS A STANDARD OBJECT FOR SURGICAL TOOLS AND ROBOTS TESTING

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Objectives: Tissue analogs are known area of research in the field of artificial organs. The ethical and scientific reasons, is going for tissue models of some properties (mechanical, electrochemical, etc.) is a wide field of applications. There is also a need for unified model of tissue for studies of new surgical instruments.
Methods: Several tissues of the cardiovascular system, describes its mechanical properties have been examined. On the other hand, common tasks, features a tool-tissue contact (touch, capturing tanks traumatic, atraumatic, tissue cutting, separation, etc.) have been developed.
Results: In order to standardize the testing of innovative tools Artificial Tissue interaction electro-Mechanical ATM model have been developed. Appropriately programmed computer controlled electromechanical system have been designed and executed. Algorithms mimic (pretending) determined tissue-tools of achieved contact have been developed. The classical instruments, laparoscopic, its own prototype tools - mechatronic and robotic RobIn Heart surgical instruments - have been studied on ATM model.
Conclusions: A unified, repeatable research station with significant opportunities to pretend to react with natural tissue has been achieved. The ATM station is currently being used in the research of innovative surgical instruments developed at the Laboratory of Biocybernetics FCSD.

P248 (EI0259)

ROBOT AS AN ARTIFICIAL SURGEONS ORGAN FOR MINI-INVASIVE OPERATION. FROM THE ANALYSIS OF THE WORK THE SURGEON'S HAND TO THE ROBOTIC ARM DESIGN ROBIN HEART

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Introduction: The surgery success depends on the information, knowledge, experience and capabilities of the surgeon's precision motion. Leaving in the

hands and brain of the surgeon the guide and decision on the telemanipulator are examples of artificial organs dedicated to specific tasks. Mini-invasive surgery provides poorer, less intrusive information, often only visual observation of the field operations. Telemanipulators thanks to that between the hand surgeon and a tool inside a patient's body is a computer - you can create a technology and technical methods and decision support systems to improve precision for the task (removing tremors hand, scaling of movement).
Objectives: This approach to design innovative instruments introduced in the Polish surgical robot, Robin Heart, project.
Methods: The process of developing the robot starts from the determination of the tools- tissue reaction (mechanical characteristic, the forces for specific operations, dynamic analysis of tools work) and person - a tool and then man-machine contact (kinematic analysis of surgeon motion). Kinematics values of multibody system: surgeon's upper limb – the classic and thoracoscopy tool or master tool manipulator – robot in different configuration (for typical surgery action elements), were determined with the use of APAS system for the trajectory of marker observation, recording and analyzing. On this basis, some typical tasks, functions, tools, and the operator behavior have been listed and a surgical robot control console trying to optimize both the issues of ergonomics, accuracy and work have been designed. RobIn Heart Shell console includes not only ergonomic handles with microjoystics but also the advisory system and the possibility of a full visual and voice communication with the operating theater.
Results: The ergonomic and high efficiency useful RobIn Heart robot system have been constructed and successfully tested in few animal and telerobotic experiments.

P249 (EI0017)
DEVELOPMENT OF HAPTIC PERCEPTION DEVICE USING "TOUCH BLEND"
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Objectives: Surgical robots, artificial arms, and artificial legs are based on the researches to present a sense of touch (haptic sense). Various researches to express a sense of touch objectively have been carried out. These have ever presented a portion of haptic perception of a sense of pressure, and of gravity. However, it is hard to say that they could express what a complex sense of touch information is.
Methods: In the 1910's, E.B.Titchener proposed "Touch Blend" of mixed haptic elements. Based on this idea, he proposed also "Touch Pyramid" of haptic sensations, which used a sense of pressure, vibration, warmth and cold, and pain to express how the sense of touch is perceived. But it had a lot of ambiguous definitions of human haptic receptors and "Touch Pyramid". Then, it needed to examine again the ambiguous points of "Touch Blend". According to these examined results, a simplified haptic perception device was produced using a vibration motor, a peltier device, and a pressure sensor. This could choose up to three senses among a sense of pressure, vibration, strain, warmth, cold, and first pain. The senses of touch were analyzed when haptic perceptions were presented on the tip of a finger using this device. 30 persons of 20's were to answer a prepared list. To avoid the sense of sight, the experiments were carried out touching the device in a black box.
Results: As results, the device was able to present the six element senses. And it was also able to present some haptic perceptions of heat, titillate, and numb. There remained some difficulties to present other complex sense of touch information completely.
Conclusions: This basic research showed that our device will be useful in the surgical robots, the artificial arms, and the artificial legs.

Biofluid Mechanics

P250 (EI0374)
EXPERIMENTAL ANAYSIS AND COMPUTER SIMULATION OF A PLAQUE FORMATION
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Objectives: The inflammatory process starts with the penetration of low density lipoproteins (LDL) in the intima. This penetration, if too high, is followed by leucocyte recruitment in the intima. This process may participate in the formation of the fatty streak, the initial lesion of atherosclerosis and then in formation of a plaque. Quantification of parameters for LDL transport and plaque formation is a huge challenge for the medical community.
Methods: In this study, an experimental model of LDL transport on the isolated blood vessel from rabbit on high fat diet after 8 weeks is simulated numerically by using a specific model and histological data. The 3D blood flow is governed by the Navier-Stokes equations, together with the continuity equation. Mass transfer within the blood lumen and through the arterial wall is coupled with the blood flow by the convection-diffusion equation. LDL transport in lumen of the vessel is described by Kedem-Katchalsky equations. The inflammatory process is solved using three additional reaction-diffusion partial differential equations. All parameters for computer model were fitted with non-linear least square procedure.
Results: Matching of histological rabbit data is performed using 3D histological image reconstruction and 3D deformation of elastic body. Concentration of macrophages obtained by computer simulation indicates that there is a newly formed matter in the intima, especially in the 5mm region before bifurcation. Computed concentrations of labeled LDL of 13% are in good agreement with experimental results.
Conclusions: Matching of labeled LDL and macrophages concentration and location between experimental and computer model shows a potential benefit for future prediction of this complex process using computer modeling.

P251 (EI0296)
SAFETY AND EFFICACY STUDY OF A NOVEL HIGH VACUUM CHEST DRAINAGE SYSTEM
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Objectives: To assess safety and efficacy of a novel high-vacuum chest drainage system (HVCDS) compared to a conventional chest drainage system (CCDS).
Methods: Four anesthetized 40kg pigs underwent a median sternotomy. Three drains were placed in retro-cardiac, retro-sternal and left pleural positions. Two animals received HVCDS (22Fr with 180 2mm-holes; Medela AG, CH), the remaining CCDS (28Fr-Atrium). After chest closure the animals had three 30-min runs of artificial bleeding by infusion of citrate blood (4mL/min) under different aspiration pressures (2,20,40kPa) for both groups, followed by a standardized surgical bleeding (40kPa-HVCDS, 2kPa-CCDS). The output of all drains, as well as the hemodynamic parameters, were registered every 5 minutes. The amount of residual blood was also measured. After euthanasia all drains, the heart and left lung underwent macroscopic and/or histo-pathological examination.
Results: The application of the different pressures showed neither hemodynamic changes nor differences in blood drainage with both systems during blood infusion and surgical bleeding. HVCDS had a trend to lower residual post-drainage blood: 10(0-15)mL vs. 16(5-25)mL (p=0.051). However, all HVCDS catheters showed relevant clotting. Application of 20kPa and 40kPa caused macroscopic epicardial and pulmonary lesions (chest tube holes) in both systems. Sub-epicardial or sub-pleural hemorrhage (depth 0.7mm) without myocyte or alveolar damage was found by histology.

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Conclusions: Both systems showed adequate equal drainage, despite marked chest tube clotting in HVCDS. High-pressure drainage with both systems showed focal sub-epicardial and sub-pleural bleeding. Thus, the long-term assessment of high-pressure chest drainage and potential interaction with fragile structure such as CABG graft should be carried out.

P252 (EI0256)
PERFORMANCE EVALUATION OF RHEO-MICROSCOPE IMAGE PROCESSING SOFTWARE FOR THE STUDY OF ERYTHROCYTE AGGREGATION
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Objectives: Aggregation properties of red blood cells (RBCs) play a key role in the circulation, contributing to the hemorheological profile. In this study we have evaluated original image processing software for studying RBCs aggregation, in view of improving the characterization of blood diseases associated with hemorheological abnormalities.
Methods: Whole blood was subjected to increasing shear rates (SR=1 to 250s^{-1}) at 37°C and simultaneously imaged with a rheo-microscope (Anton Paar), comprising a parallel-plate rheometer and an optical microscope (20x). A video sequence of the flowing erythrocytes was acquired during each test, and subsequently analyzed. A custom software for image segmentation, previously designed and validated by the authors, was used to determine, for each frame, the number and size of RBC clusters.
Results: Normal RBCs at rest or under low flow can be found as aggregates. An increase in SR causes their progressive fragmentation. The software tracked the total area of the aggregates, which can drift along the experiment and must be normalized by the cluster number. At moderate SR, the number of new RBC clusters per unit of time (dN/dt) was seen to increase, from 0.66 clusters/s at 50s^{-1}, to 1.18 clusters/s at 100s^{-1}. Correspondingly, the temporal gradient of mean cluster area (dA_{mean}/dt) was -26.48μm^2/s and -25.13μm^2/s, respectively (i.e., approximately 1 RBC lost every 2 seconds). Actually, dN/dt and d(A_{mean})/dt have an opposite theoretical dependence on the number of clusters (N), whereas their product absolute value is weakly dependent on N, and instead increases with SR, exactly as hereby observed. Finally, complete disaggregation occurred at SR=200s^{-1}.
Conclusions: Image analysis of RBCs was found to be capable to track automatically erythrocytes aggregation properties. Direct calculation of geometric features of RBC clusters is feasible, without resorting to indirect measurements such as, e.g., those based on backscattered light.

P253 (EI0195)
PASSIVE FLOW REGULATION IN CAPILLARY NETWORKS
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Objectives: Capillary blood flow is a two-phase flow: blood cells and plasma are moving through a network. Which forces shape this flow? How are the cells distributed? What are the regulation mechanisms? This is investigated experimentally with an enlarged model, which is made to simulate the deformability of the red blood cells and the resulting effects on flow resistance. This experimental model was developed because in experiments with real blood the network is too minute and detailed numerical simulation is too complex.
Methods: We use a blood model that consists of water droplets of about 1mm diameter (red blood cells) and sunflower oil (plasma). Two syringe pumps pump the components into a bifurcation, where the droplets are formed. The model is applied to flow through bifurcations and through a network. The network model is made of transparent silicone rubber. It consists of capillary segments, whose diameters and lengths are statistically similar to the pulmonary capillary network of rats, but enlarged to an average diameter of 1.2mm.
Results: The experiments in the capillary network model show a droplet distribution which is variable, especially in segments with lower droplet fraction. A segment holds only a small number of droplets. Hence, a segmental flow resistance changes considerably when a droplet enters it or leaves it. Consequently, the flow distribution changes, which acts back on the number of droplets entering the segment - an unsteady flow is induced.
Conclusions: Effects seen in both systems – *in vivo* and in the model – must be essentially influenced by the properties they have in common. This means, the variable hematocrit seen in pulmonary networks is at least in part a passive mechanism, induced by the flow resistance change due to the red blood cells and the droplets, respectively.

P254 (EI0110)
A COMPUTATIONAL MODEL OF PULSATILE FLOW PAST AN OSCILLATING CYLINDER
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Objectives: A fundamental study to characterize the flow around an oscillating cylinder in a pulsatile flow environment is investigated. This work is motivated by a new proposed design of the Total Artificial Lung (TAL), which is envisioned to provide better gas exchange.
Methods: Cylinder oscillations and pulsatile free stream velocity were represented by two sinusoidal waves with amplitudes A and B and frequencies ω_c and ω, respectively. The average free stream velocity is U_0. A Newtonian fluid was considered and the governing Navier-Stokes equation was solved using the finite volume method.
Results: It was observed that an increase in amplitude and a decrease in the Keulegan-Carpenter number ($K_c=U_0/D\omega_c$) are associated with an increase in vorticity (up to 246%) for every Reynolds number (5<Re<20) suggesting that mixing could be enhanced by the proposed TAL design. The drag coefficient was found to decrease for higher amplitudes and lower K_c for all cases investigated. In some cases the drag coefficient values were found to be lower than the stationary cylinder values (A=0.5, K_c=0.3 and Re=10 & 20). A "*lock-in*" phenomenon (cylinder oscillating frequency matched the vortex shedding frequency) was found when K_c=1 for all cases. This lock-in condition was attributed to be the cause of the rise in drag observed in that operating regime.
Conclusions: The results suggest that operating the TAL at higher fiber oscillation amplitudes and lower K_c (avoiding the lock-in regime) may provide optimal performance of the modified TAL design.

General

P255 (EI0361)
APPLICATION OF MULTI-WALLED CARBON NANOTUBES IN THERMAL TREAMENT OF CANCER
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Objectives: To investigate the ability of multi-walled carbon nanotubes (MWCNT) in absorbing the near infrared light for the treatment of cancer.
Methods: Colorectal cancer SW620 and HT29 were seeded into two separate 96-well plates for 24 hours. A group of cells were then incubated with variable concentrations of functionalised MWCNT (0.1mg/mL, 0.2mg/mL and 0.3mg/mL) for 24h. The wells were then exposed to 808nm laser light with power of 1W/Cm^2 at different time intervals (1, 2, 3, 4 and 5 minutes). Cell viability for cells with MWCNT, laser or in combination was measured and compared with cells without MWCNT and laser, using variable techniques such as Alamar blue, Trypan blue and Fluorescence Activated Cell Sorting (FACS).
Results: Results suggest that in the group of cells that were treated with both laser and MWCNT, by increasing the concentration of MWCNT and the timing of exposing the laser to the cancer cell, the number of cell death significantly increases. The cells that were treated with MWCNT without laser maintained high cell viability, similar to untreated sample. The results also show that in the case of laser alone, by increasing the duration of exposure of laser on the cell the number of cell death increase. However this is occurring with a lower magnitude in comparison to the combination of laser and the MWCNT.
Conclusions: MWCNT has the ability of absorbing the near infrared wavelength. This property of MWCNT can be used for the thermal treatment of cancer.

P256 (EI0298)
BIOLOGICAL TISSUE AND METAL MATERIAL ADHESION TECHNOLOGY USING INTEGRATED LOW-LEVEL ENERGIES
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Objectives: We have proposed a new adhesion method by using integrated low-level energy sources. This adhesion method is expected to be applied

to adhere biological tissue to the metal, such as stent-grafts, synthetic blood vessel and blood inlet cannula of ventricular assist devices. As fundamental research to adhere biological tissue to biocompatible metal material, we examined the relationship between adhesion performance and surface roughness of metal material.
Methods: Shear tensile tests on slices of porcine aorta adhered to a metal specimen were performed to determine the relationship between adhesive strength and surface roughness of the metal specimen. The metal materials used for specimen was stainless steel, which is extensively used as biocompatible material. The surface of metal specimen was roughened up with sandblast. The roughness of original surface metal specimen was an Ra of 0.05μm. Surface roughness of sandblasted metal specimens ranged from an Ra of 0.25μm to an Ra of 0.80μm.
Results: Biological specimens can be adhered to a metal specimen. The adhesive strength of the biological specimen to the original metal specimen at an Ra of 0.05μm was 0.25MPa, and is higher than aldehyde glue adhesive strength that is 0.01MPa. The average strength of surface roughens specimens ranged from 0.35MPa to 0.45MPa and is higher than that of the smooth original specimen.
Conclusions: We proposed a new method to adhere biological tissue to metal material using integrated low-level energy sources. The adhesive performance was found to be improved by roughening the surface of the metal specimen. Our adhesion method is less invasive, has a more powerful adhesive strength than aldehyde glue.

P257 (EI0118)
A NEW METHOD TO QUANTIFY EYE-BLINK RESTORATION IN FACIAL PARALYSIS
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Objectives: Electrical stimulation (ES) of the paralyzed eyelid, triggered by the corresponding function on the contralateral healthy side, has been proposed to treat eyelid closure impairment in unilateral facial paralysis. However, results in terms of functional and cosmetically acceptable eyelid closure have been poorly documented. We propose a new method to quantify the effective restoration of the blink provided by a prototypal device developed for a contralaterally elicited eyelid ES.
Methods: On a healthy subject surface EMG electrodes were used to detect the natural eyeblink (N-blink) on one side and to trigger an electrical stimulation leading to an artificial blink (A-blink) of the orbicularis oculi muscle on the controlateral side. Trains of 10 pulses (4mA amplitude, 2ms duration) were delivered at various frequencies: 50, 100, 150, 250Hz. Assuming that during the eyeblink the eyelid rotates around the axis passing through eye canthi, a miniature gyroscopic sensor was used to estimate maximum eyelid motion from open eye to complete closure (CC) and eye-blink duration (D). Both the N-blink and A-blinks evoked by the different patterns of stimuli were measured and compared.
Results: The N-blink was characterized by a CC of 3.5mm and D of 380ms. Stimulation at 50 and 100Hz showed distinct differences from the N-blink: 50Hz train caused ineffective eyelid closure (CC= -41%) and an excessive eyeblink duration (D=+215%), while 100Hz train showed a more complete eyelid closure (CC=+6%) but longer eyeblink duration (D=+67%). Conversely, 150Hz train and 250Hz train provided a quite natural-like A-blinks (CC=+10%,+11%; D=+14%,-15%, respectively).
Conclusions: The new gyroscopic-based method showed to be a valuable tool to quantify the effective and natural-like eyeblink restoration provided by the EMG–triggered ES device. Further studies will be necessary to evaluate the method in facial paralysis patients and to also provide quantification of potential alterations of facial mimicry associated with ES.

P258 (EI0105)
LIPOSOME-ENCAPSULATED HEMOGLOBIN ENHANCES CHEMOTHERAPY TO SUPPRESS TUMOR GROWTH AND METASTASIS IN MICE
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Objectives: Liposome-encapsulated hemoglobin (LEH) with high O_2-affinity ($P_{50}O_2$=10 mmHg, h-LEH) has been reported to enhance tumor radiosensitivity. Anticancer drugs, such as doxorubicin (DXR) but not 5-FU, require O_2 to be cytotoxic as in radiotherapy. We hypothesize that targeted O_2 delivery to tumor hypoxia by h-LEH may enhance chemotherapy and evaluated effects of h-LEH added to chemotherapy on tumor growth and metastasis in mice.
Methods: DXR and S-1 (a novel oral 5-FU derivative) were applied on the Lewis Lung Carcinoma (LLC) grown in the leg of C57BL/6N mice. Daily administration of DXR (0.5mg/kg, intraperitoneally) or S-1 (8mg/kg, orally) was started 48h after intramuscular inoculation of LLC for 2 consecutive weeks. H-LEH (5 mL/kg) was intravenously infused 2h after each chemotherapy every other day for 2 weeks. After these 2W treatments, mice were sacrificed for quantitative and macroscopical examinations of the tumor growth and lung metastasis.
Results: The administration of h-LEH (5 mL/kg) or DXR (0.5 mg/kg) alone had no effect on tumor growth in the leg and metastasis in the lung. The addition of h-LEH to DXR resulted in 30.5% reduction of tumor weight (P<0.05) and 41% reduction of lung metastasis (P<0.01). While S-1 had a marked effect on both tumor growth (35% tumor weight reduction) and 62% reduction of metastasis, the addition of h-LEH had no synergistic effect on the anti-tumor effect of S-1.
Conclusions: These results suggest that h-LEH may be effective to enhance chemotherapeutic agents that require tumor oxygenation to suppress tumor growth and lung metastasis in mice.