
Laser therapy for cystic fibrosis-associated infection

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Introduction. One of the major hallmarks in cystic fibrosis epithelium is exacerbated inflammation, accompanied by suppressed ability to clear pathogens. Indeed, the vast majority of these patients become infected with opportunistic pathogens, such as *Pseudomonas Aeruginosa*, that often become resistant to multiple antibiotics. Chronic bacterial pulmonary infections, combined with exaggerated inflammation, cause a progressive decline in lung function, which represents the main cause of morbidity and mortality in cystic fibrosis patients.

Aim. To assess the anti-microbial activity of different blue laser protocols on *Pseudomonas Aeruginosa* biofilms.

Materials and methods. *Pseudomonas Aeruginosa* were grown as biofilms, on both glass slides and plastic plates, with a liquid layer covering the biofilm. Subsequently the bacterial biofilms were irradiated with a blue laser using two different protocols: 300mW/cm², 100J, 300sec (Tr1) and 600mW/cm², 200J, 600sec (Tr2). Bacterial growth was assessed in Tr1, Tr2 and control groups 6 hours later. Moreover, we have also largely worked on the technological aspect, developing a new prototype of diode laser specifically, created by K-Laser Company, equipped with a mechanical machine, conveniently designed to provide uniform irradiation to different multiwell plates (12, 24 and 96 plates).

Results. Both Tr1 and Tr2 treatments significantly reduced cell viability and bacterial growth compared to the control group. The new prototype used for the present study allowed a uniform irradiation, with a minimum difference, in term of standard deviation, between wells belonging to the same group.

Conclusion. Both Tr1 and Tr2 blue laser protocols showed a marked and reproducible anti-microbial effect on *Pseudomonas Aeruginosa* biofilms. Due to its anti-microbial activity, the blue laser could facilitate the penetration of antibiotic drugs into a bacterial biofilm in cystic fibrosis patients.

References

- Hoiby N. Understanding bacterial biofilms in patients with cystic fibrosis: current and innovative approaches to potential therapies. *J Cyst Fibros.* 2002;1:249-254.
- Kadurugamuwa JL, et al. Direct continuous method for monitoring biofilm infection in a mouse model. *Infect Immun.* 2003;71:882-890.
- Ottaviani G, et al. Effect of class IV laser therapy on chemotherapy-induced oral mucositis: a clinical and experimental study. *Am J Pathol.* 183:1747-1757.

The role of the innate immune response in HPV-related oral and oropharyngeal cancer

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Introduction. During the last 20 years, the incidence of HPV-associated oropharyngeal cancer is increased. Principal actors of the innate immune response against HPV are represented by the TLRs (Toll like receptors). On the other hand different studies have reported that HPV can directly inhibit the functions of the TLRs pathway through interferons (IFNs). There are very few preliminary studies on the role of TLRs mediated HPV clearance in human oncology. Our study aim has been to evaluate whether TLR4 identifies HR-HPV integration state in OSCC.

Methods. Protein levels of TLR4 in OSCC were assessed using Immunohistochemistry (IHC). *In situ* hybridization (ISH) for HPV-DNA detection in morphological context and Pyro-sequencing method have been performed in order to detect viral integration or episomic status. The relationship between TLR expression with or without HPV infection has been elucidated.

Results. ISH HPV positive samples have reported lower TLR4 intensity than negative samples and it has confirmed by statistically significant difference ($p = .002$). There is no statistical correlation between TLR4 intensity and PCR HPV results ($p > 0.05$). Point-biserial correlation coefficient revealed statistically significant association between TLR4 expression and HR-HPV integration status ($p = .0001$) and between TLR4 expression index and HR-HPV infection ($p = .001$).

Conclusions. We retain that TLR4 down-regulation is not associated to the histological tumoral grade but rather to HPV-16 infection and to its integration state into the host DNA.

Graphene coated hydroxiapatite scaffolds for bone regeneration: preparation and characterization

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Objectives. Currently, an extraordinary interest is seen to explore the potential of graphene for applications in biomedical and regenerative engineering. The aim of the present study was to design, prepare and characterize new hybrid graphene-hydroxyapatite (HA) based scaffolds for bone regeneration.

Methods. The materials, porcine bone derived HA (Apatos, OsteoBiol®, Tecross, Coazze, Italy) and equine bone derived HA added with collagen (Gen-Os, OsteoBiol®, Tecross, Coazze, Italy) were developed by coating HA granules with graphene oxide (GO), prepared by a modified version Hummers method. Graphene samples were characterized by Raman, SEM, TEM and AFM spectroscopy. *In vitro* MTT analysis, indicative of cellular metabolic activity, was performed on human gingival fibroblasts (HGF) cultured on the hybrid prototypes.

Results. Preliminary TEM measurements highlight a good exfoliation of the different graphene derivatives, with the majority of graphene samples composed of one to three layers and an hybrid characterized by a good and homogeneous coating of the HA granules, as confirmed by Raman and other microscopy measurements. *In vitro* preliminary MTT analysis on HGF shows no toxic effects of GO, whereas GO-enriched HA improves the metabolic cellular response when compared to HA alone up to 7 days of culture.

Conclusions. The obtained materials appear to be very promising for regenerative engineering applications. Further investigations will be conducted to assess the osteoinductive potential of the newly developed materials.

References

- Gu M, Liu Y, Chen T, Du F, Zhao X, Xiong C, Zhou Y. Is Graphene a Promising Nano-Material for Promoting Surface Modification of Implants or Scaffold Materials in Bone Tissue Engineering? *Tissue Eng Part B Rev.* 2014 Feb 27.
- Mena F, Abdelghani A, Mena B. Graphene nanomaterials as biocompatible and conductive scaffolds for stem cells: impact for tissue engineering and regenerative medicine. *J Tissue Eng Regen Med.* 2014 Jun 11.

Evaluation of BP180 and BP230 ELISA in the diagnosis of mucous membrane pemphigoid limited to the oral cavity: preliminary results

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Objectives. Mucous Membrane Pemphigoid (MMP) is the clinical phenotype of a group of rare autoimmune blistering diseases characterized by autoantibodies directed against different structural proteins in epithelial basement membranes. Diagnoses of MMP is routinely verified by direct immunofluorescence (DIF) of oral mucosa biopsy tissue. ELISA detection of autoantibodies in serum is now employed for the diagnosis of pemphigoid and for monitoring the disease activity. The aim of this study was to evaluate ELISA sensitivity, specificity, PPV, NPV in oral MMP patients.

Methods. Patients with oral lesions compatible with MMP, were enrolled. Two different specimens were obtained during the surgical biopsy: one for the histopathological assessment and the other for the DIF. ELISA plates, precoat-