



3<sup>o</sup> Meeting

IBIM-CNR



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UNIVERSITÀ  
DEGLI STUDI  
DI PALERMO

BIOINFORMATICA IMMUNOLOGIA  
MALATTIE APPARATO RESPIRATORIO  
MALATTIE METABOLICHE  
MICROORGANISMI NELLE BIOTECNOLOGIE  
NANOTECNOLOGIE NEUROSCIENZE  
ONCOLOGIA SVILUPPO E DIFFERENZIAMENTO

# LIBRO degli ABSTRACT



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effective carrier to transport efficiently therapeutics to the posterior eye segment, minimizing drug loss and side effects. However, being the cornea the most important anatomical barrier that limits the delivery of drugs into the eye, the evaluation of permeation through this barrier is necessary. The aim of this preliminary study is to evaluate an ex vivo model useful to study the permeation of drugs and above all nanotechnology-based drug delivery systems across the cornea. This model implies the use of bovine corneas, as one of the most useful model to simulate human corneas, and Franz type diffusion cells. This should be used to evaluate the capacity of nanotechnology-based drug delivery systems to enhance and promote the entrance of drug into the eye. In particular, the use of polymeric micelles based on polysaccharide polymers is proposed. New inulin (INU) and hyaluronic acid (HA) amphiphilic derivatives (INU-EDA-RA and HA-C16) were synthesized. In addition, dexamethasone was chosen as an effective drug useful for retinal diseases. Consequently, dexamethasone loaded INU-EDA-RA and HA-C16 micelles were prepared and characterized.

## MM6

### Anti-inflammatory effects of formoterol and fluticasone propionate in bronchial epithelial cells

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**Body:** The addition of long-acting  $\beta_2$ -agonists (LABAs) to corticosteroids improves asthma control. Cigarette smoke exposure increasing oxidative stress, increases airway inflammation and may negatively affect corticosteroid responses. The anti-inflammatory effects of formoterol (FO) and fluticasone propionate (FP) in human bronchial epithelial cells (16HBE) exposed to cigarette smoke extracts (CSE) are unknown.

**Aims:** This study was aimed to explore whether FO combined with FP, counteracts some CSE-mediated effects in 16HBE including: 1) the nuclear translocation of Glucocorticoid Receptor (GR); 2) the nuclear expression of NF-KB; 3) the expression of the NF-KB related cytokines IL-8 and TNF $\alpha$ . **Methods:** 16HBE were stimulated with CSE and/or FO and FP. Nuclear translocation of GR and NF-KB were assessed by western-blot analysis. IL-8 and TNF $\alpha$  expression were evaluated by Real-time PCR. **Results:** CSE decreased the expression of GR and increased the expression of nuclear NF-KB. FO combined with FP, was able to revert these phenomena in CSE stimulated 16HBE cells increasing the nuclear translocation of GR and decreasing the nuclear translocation of NF-KB. FO combined with FP reduced the expression of IL-8 and TNF $\alpha$  in CSE stimulated epithelial bronchial cells. **Conclusions:** The present study provides compelling evidences that FO may contribute to revert some processes induced by oxidative stress and is able to increase the anti-inflammatory effects of FP.

## MM7

### Different modulatory effect of the synthetic cannabinoid WIN55,212-2 on tumor cell migration

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MicroRNAs are small non-coding regulatory molecules exerting pleiotropic action in different biological processes such as proliferation, differentiation, apoptosis, migration and metastasis. Deregulation of miRNA expression has been observed in various cancers, and accumulating data suggest that miRNAs can display an oncogenic, antioncogenic or an ambiguous behavior in relationship to tumor environment. In a previous

research we showed that the synthetic cannabinoid WIN55,212-2 is able to reduce the migratory activity of osteosarcoma MG63 cells analyzed by means of wound healing assay. So we undertook a study to evaluate the biochemical mechanism through which WIN plays this action. To this purpose we evaluated the levels of miR-29b1, a member of miR-29 family which has been shown to impact critical steps in the migratory and metastatic cascade, such as EMT, apoptosis and angiogenesis. RT-PCR experiments showed that in MG63 cells 5 mM WIN increased the level of miR-29b1 of about 700-fold. This effect was accompanied by the reduction in its putative targets MMP-2, PDGF-B and N-MYC, thus indicating that the miRNA is functionally active. Moreover, cells stably overexpressing miR-29b1 did not close the wound after 48 h, mimicking the effect of WIN in untransfected control cells. Notably, ER $\alpha$ (+) MCF-7 and triple negative MDA-MB-231 cells, two different breast cancer models, treated with the cannabinoid migrated into the scratched area significantly faster than the respective control cells. In these cells WIN also increased the level of miR-29b1 targets. Therefore, differently from osteosarcoma cells, these preliminary observations seem to indicate that WIN promotes migration ability in breast cancer cells. The reasons for this diverse behaviour could rely on miR-29b1, whose expression can change in different cell types or show temporal differences dictated by cell physiology and tumor microenvironment impact. Studies are in progress to shed light on the molecular mechanisms underlying this different response.

## MM8

### **A study of cell-cell fusion through generation and culture of osteoclasts from RAW 264.7 cells**

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One of the unique ability of macrophages is to fuse each other to form multinucleate giant cells (MGCs). The most characterized type of MGCs are the osteoclasts (OCs). Two osteoblast-derived cytokines, M-CSF and RANKL, control osteoclastogenesis through engagement of their cognate receptors c-fms and RANK, respectively. RAW264.7 macrophages are considered pre-OCs and become OCs within 4-5 days after stimulation with RANKL. They respond recruiting TRAF6, a receptor-associated factor, which triggers a cascade of transcription factors, including NF- $\kappa$ B, NFATc1 and c-Fos. These factors switch on the expression of OC-specific genes, such as those coding for the enzymes TRAP and cathepsin K, and the fusion-specific molecules DC-STAMP and ATP6v0d2. Ultimately, pre-OCs TRAP+ fuse to form multinucleated mature OCs TRAP+. Dynamic reorganization of the cytoskeleton mediates cell fusion, through drastic and regular variations of filopodia and podosomes. In this study, by means of IF, we found that all mononuclear cells showed podosomes 1 day after RANKL stimulation. At the same time, RANKL treatment inhibited phagocytic ability of RAW 264.7 cells dose-dependently, suggesting a decrease of macrophages and a gain of pre-OCs properties. After 3 days from RANKL addition, RAW264.7 cells started to form cell aggregates. The number and density of podosomes, which appeared as dots on pre-OCs, increased during OC maturation. Some cells in the aggregates possessed podosomes assembled into small actin rings, which eventually formed podosome belts on the 4<sup>th</sup> day. Multinucleated TRAP+ cells, i.e. exhibiting TRAP activity as intense cytoplasmic red staining under light microscopy, were considered as differentiated OCs. Electron microscopy images showed 4-days treated cells at various steps of cell fusion. Canonical NF- $\kappa$ B pathway was induced rapidly in pre-OCs in response to RANKL and, by means of IF, we found that a second peak of induction for NF- $\kappa$ B signaling is required for terminal differentiation of pre-OCs.





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