HYPER-OSMOLARITY AND DRYNESS MODEL ON HCE

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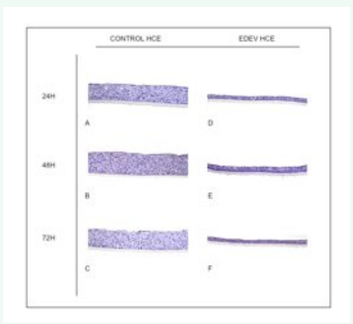
BACKGROUND

Tear Dysfunction Syndrome (TDS) or Dry Eye Disease is a multifactorial disease of the tears and ocular surface that results in symptoms of discomfort and tear film composition modification and instability with potential damage to the ocular surface. Literature reports that tears from patients affected by the TDS are hyper-osmolar determining a further stress to the corneal epithelium.The corneal epithelium, a non keratinized multilayered squamous epithelium of about 60 μm, has a crucial role in the barrier function and can be considered the first line of defence against many types of injury, trauma or infection. Furthermore, the corneal epithelium is directly involved in the establishment of dry eye with discomfort and pain associated to the dry eye syndrome.A dry-eye non animal model that mimics the human dry-eye disease would be a useful tool for investigating the multiple factors that have been implicated in the pathogenesis of TDS.

We have published an experimentally induced dry eye *in vitro* model (EDEV) on an in vitro human corneal epithelium (HCE) model in order to induce the most relevant morphological, cellular and molecular modifications related to the dry eye symptoms: inflammation, modification of the structural compartments of ocular surface and microvilli "network". In order to complete the model in this work are reported the preliminary results associated to hyper-osmolarity conditions by treatment with Sodium Chloride and Sorbitol.

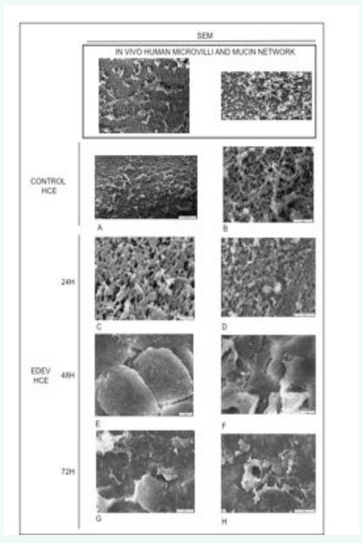
RESULTS

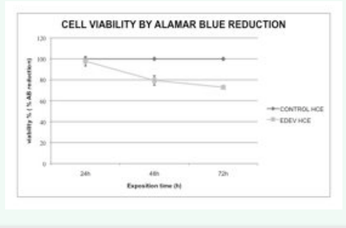
EDEV MODEL



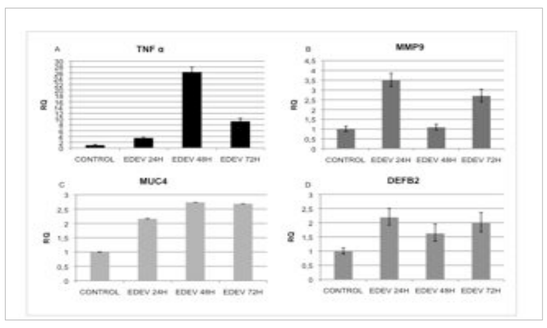
A dramatic reduction in the thickness of the epithelium was observed in EDEV-HCE compared to CONTROL-HCE tissue.

At 24 h post-induction, the number of microvilli was reduced; microvilli were no longer present at 48 h and 72 h, and the desiccating stress was apparent on the epithelium surface.
In the EDEV condition the tissue integrity was no longer observed





After 72 h, the survival of HCE in EDEV conditions was still 70% compared with CONTROL-HCE



TNF-*α* expression was maximal at 48 h. Over-expression of MMP-9 was observed at 24 h and 72 h following EDEV induction.High levels of MUC4 expression were observed at all time points analyzed.
A moderate increase in the expression of DEFB2 was observed at 24 h and 72 h after dry eye induction. maximal at 48 h

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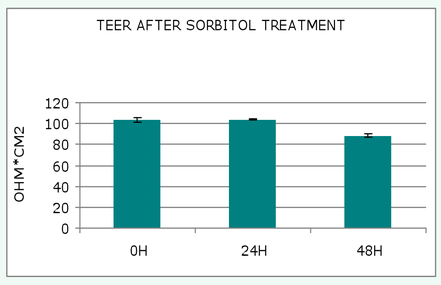
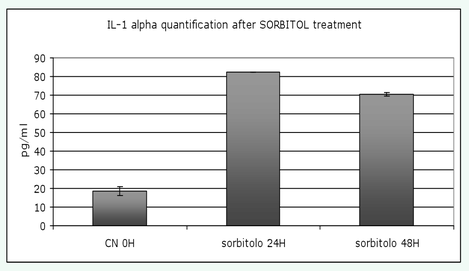
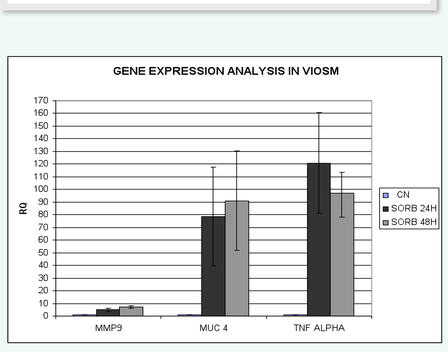
HYPER-OSMOLARITY MODEL





SORBITOL 24H

SORBITOL 48H



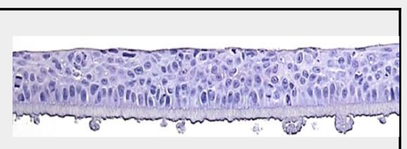
Tears of patients with chronic dry eye contained increased amounts of proinflammatory cytokines and chemokines and increased amounts of MMP-9 than in normal tears.

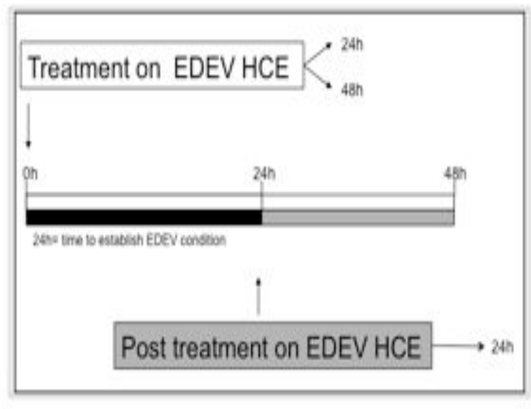
Desiccating stress increased expression of released proinflammatory cytokine IL-1α and mRNA for TNF-*α*, MMP-9 and MUC4 in corneal epithelium *in vivo*.

In our **hyper-osmolarity model after 24h** treatment with sorbitol the release of **IL-1**α has been quantified as significantly higher than in the control HCE, the Trans Epithelial Electrical Resistance -**TEER** was not modified and the **MMP-9, MUC-4 and TNF-*α*** genes have been up-regulated.

HUMAN OCULAR SURFACE		
0.01-0.1 µm	LIPIDS	
4-10 µm	WATER	
	MUCINES	
60-70 µm	CORNEAL EPITHELIUM	

EXPERIMENTAL APPROACH

DRY EYE MODEL	**In Vitro Reconstructed Human Corneal Epithelium (HCE) SkinEthic –lyon (F)** 	
PROTOCOL	**HYPER-OSMOLARITY** Hyper-osmolarity induced by Sodium Chloride or Sorbitol added in the culture medium for 24h up to 48h	**EDEV** Dry eye induced by controlled environmental setting (RH<40% and T=40°C) for 24h up to 72h
PARAMETER	1) gene expression by using TaqMan gene assay technology (*MUC4*, *MMP9*, *TNF-α*) 2) trans-epitelial electrical resistance (TEER) 3) IL-1*α quantification*	1) gene expression by using TaqMan gene assay technology (*MUC4*, *MMP9*, *TNF-α*, and *DEFB2*) 2) Alamar blue assay, Histology and immunohistochemistry staining 3) Scanning electron microscopy



DISCUSSION

The **EDEV HC**E model stimulated the production of inflammatory factors TNF -α and MMP-9 :*in vivo* in the dry eye increased production and activation of pro-inflammatory cytokines and proteolytic enzymes by stressed ocular surface have been reported.Increased levels of MMP9 were also observed in EDEV HCE after 24h and 72h of dryness stress : *in vivo* increased levels of proinflammatory cytokines and metalloproteinases have been observed on the ocular surface of patients with kerato-conjunctivitis secca. Two fold induction of MUC4 mRNA was found in EDEV HCE at 24h, 48h and 72h: *in vivo* MUC4 is considered a membrane-bound mucin and is secreted by conjunctival and corneal epithelial cells and ocular mucus protects against bacterial adherence to the corneal epithelium and alteration in mucus production promote bacterial adherence to the cornea.

In vivo the ocular surface in dry eye disease is compromised and therefore at risk for microbial infections : while an increased expression of hBD-2 may be beneficial in terms of antimicrobial protection, it may also contribute to ocular surface damage observed in subjects with dry eye : in our EDEV *in vitro* model a low but significant over-expression of beta defensin-2 has been quantified. The hyper-osmolarity condition was obtained successfully by adding sorbitol, an osmotic stressor, in the medium underneath the tissues and the preliminary results obtained on physical, biochemical and molecular parameters nicely describe the stress condition observed *in vivo.* By using a dynamic approach, we were able to define a set of biomarkers gene signatures of dry eye-induced effects highly predictive of corneal damage i*n vivo.*

REFERENCES
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