INTRODUCTION

• Damage to the corneal epithelium caused by chemical, physical or microbial insults results in swelling of the stroma, atrophy of stromal fibroblasts and infiltration of various inflammatory cells, leading to loss of corneal transparency and integrity. Therefore, it is important to repair corneal epithelial damage as rapidly as possible. Most corneal epithelial wounds are indeed repaired promptly. However, under certain clinical conditions, treatment of the corneal epithelium is delayed. Knowledge of the mechanisms that underlie the healing of corneal epithelial wounds is crucial for the development of new treatments.

• Reepithelialization of corneal epithelial defects occurs in three phases: 1) immediately after the wounding of epithelial cells, the neighboring intact epithelial cells begin to migrate over the affected area until they cover it with a cell monolayer. 2) This is followed by the proliferation of the migrated epithelial cells, resulting in the restoration of the normal epithelial thickness. 3) A few weeks after the wounding occurred, the epithelial cells begin to differentiate, the surface of the healed region becomes smooth and a well-structured layer is restored. The three phases of corneal epithelial wound healing can therefore be characterized by migration, cell proliferation, and cell differentiation, respectively.

• Two different types of epithelial movement during the covering process of the exposed area of the cornea have been observed as shown in Fig. 1: epithelial abrasion (upper panel), followed by an initial sheet-like movement of individual epithelial cells (middle panel) and a subsequent lamellipodium-like movement of the remaining epithelial cells (lower panel).

Fig. 1: Hepatitis movement

Immediately after epithelial abrasion
Sheel-like movement
Lamellipodium-like movement

• The matrix metalloproteinases (MMPs) are a family of proteolytic enzymes whose function is to maintain and remodel the tissue architecture. They have been implicated in a wide range of processes and diseases and their expression is upregulated in wound fibroblasts by autocrine loop for long term remodeling.

• MMP-9, the primary MMP synthesized and secreted by basal corneal epithelial cells during the healing process, was shown to be upregulated in xanthan gum treatment groups. At 72 h the levels of expression of MMP-9 in the hyaluronic acid treated group were doubled compared to the positive control (dexamethasone), while a 3-times increase was observed in the xan

Fig. 2: MMPs in Corneal Wound Healing

BIOLOGICAL MODEL

Commercially available 3D HCE multilayer of 0.5 cm in diameter were cultured in chemically defined medium, modified MCDB 153 without added serum, was supplied by SkinEthic and it was used for the experiment. Epithelial corneal cells were deposited on a polycarbonate filter and cultured at the air-liquid interface for 7 days in a chemically defined medium (modified MCDB 153) in order to form a stratified epithelium.

This model has been fully characterized and it presents the same features and morphology of the human corneal epithelium in vivo (66-70µ thick- ness) (Fig. 3).

Injury and treatments:

Reproducible superficial incisions were obtained by using a sterile thin glass edge and by making 4 incisions on the middle of the HCE surface. HCEs were placed on 6 well plates with culture medium and, immediately after the injury, all the HCEs were placed in a CO2 incubator (95%) at 37°C. The maintenance me-

medium, modified MCDB 153 without added serum, was supplied by SkinEthic, and it was changed every 2 h.

Thirty minutes following mechanical injury, HCE samples were treated with 1% or 0.2% xanthan gum formulations, or with 1% xanthan gum plus 0.15% sodium hyaluronate gel and were monitored at the fol-

In Fig. 4, the proliferation and migration of multi-layered cultures are quantified and compared with the control group. The proliferation and migration of multi-layered cultures are quantified and compared with the control group.

Fig. 4: Mechanical lesion

T0 T6 T48

Fig. 6: Expression of MMP9 (RT-PCR) T 72 h

MMP-9 gene expression was measured by real time RT-PCR, using commercially available primers. The mRNA levels were determined by comparing the values obtained in the treated samples with the control group. The normalized expression values were calculated using the equation: 2^(-∆∆CT). The results are expressed as mean ± S.D. of three independent experiments. The data were analyzed by ANOVA. The level of significance was set at p < 0.05.

The proliferation and migration of xanthan gum treated groups were significant compared to the control group.

Fig. 5: Treatments T 48 h

REFERENCES


With this experimental in vitro model of mechanical injury on HCE multilayer, it was possible to monitor the wound healing process and to investigate the effect of treatments. The optimal time point to highlight differences among treatments was 48 h.

The proliferation and cellular migration took place earlier when the cells were treated with xanthan gum. The tissue of best quality was obtained when the combination xanthan gum plus sodium hyaluronate was used, confirming the data obtained in in vivo studies. The xanthan gum probably improves and speeds up the healing process by promoting MMP-9 expression, known to be released by HCE to remodel the matrix behind the leading migratory front.

CONCLUSIONS

The proliferation and migration of multi-layered cultures are quantified and compared with the control group. The proliferation and migration of multi-layered cultures are quantified and compared with the control group.

Fig. 6: Expression of MMP9 (RT-PCR) T 72 h

A: Hyaluronic acid 0.15% B: Hyaluronic acid + xanthan gum 0.1% C: xanthan gum 0.1% D: xanthan gum 0.1% E: Dexamethasone, Red line, xanthan

MMP-9, the primary MMP synthesized and secreted by basal corneal epithelial cells during the healing process, was shown to be upregu-

lated in xanthan gum treatment groups. At 72 h the levels of expression of MMP-9 in the hyaluronic acid treated group were doubled compared to the positive control (dexamethasone), while a 3-times increase was observed in the xan

Fig. 5: Treatments T 48 h

0.5% Sodium Hyaluronate

0% xanthan gum

1% xanthan gum

1% xanthan gum plus 0.15% Sodium Hyaluronate

1% xanthan gum plus 0.15% Sodium Hyaluronate

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With this experimental in vitro model of mechanical injury on HCE multilayer, it was possible to monitor the wound healing process and to investigate the effect of treatments. The optimal time point to highlight differences among treatments was 48 h. The proliferation and cellular migration took place earlier when the cells were treated with xanthan gum. The tissue of best quality was obtained when the combination xanthan gum plus sodium hyaluronate was used, confirming the data obtained in in vivo studies. The xanthan gum probably improves and speeds up the healing process by promoting MMP-9 expression, known to be released by HCE to remodel the matrix behind the leading migratory front.

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