Ocular Diseases

KERATOCONUS

InViMOS focus

Background

Keratoconus (KC) is characterized by thinning and protrusion of the cornea, resulting in an irregular, conical shape, which leads to severe vision impairment. The abnormalities in KC include the degeneration of epithelial basal cells and breaks in Bowman's layer, as well as the release of catabolic enzymes and cytokines that cause thinning of collagen matrix lamella and apoptosis of corneal keratocytes (Hollingswmorth JG, 2005). The extreme thinning of stroma appear to be related to increased proteinase activity, along with decreased proteinase inhibitors. In addition, in the stroma keratocytes decrease due to activation of apoptotic pathway and inhibition of proliferation, and fibrils are compacted and lose their arrangement and collagen lamellae numbers decrease.

The KC pathogenesis still unclear, however, a variety of factors have been considered, including genetics and cellular mechanisms, as well as oxidative stress. Recent studies demonstrated increased levels of pro-inflammatory cytokines in KC tear film, suggesting the involvement of chronic inflammatory events in the disease.

Pathology Model

1. **Human Corneal fibroblasts** – Tissue repair and wound healing in the cornea is accomplished mainly by the corneal fibroblasts, and these cells are responsible for pathological process in the degenerative corneal disorders such as keratoconus. In our model, human corneal fibroblasts (HCF) are challenged with stressful conditions, either a combination of IL6 and TNFalpha, or human tears from moderate keratoconus patients (InViMOS platform), or low-pH conditions.
2. **Human epithelial cells** – The modulation of oxidative stress at the ocular surface by Client's compounds will be tested on a model of corneal surface, constituted by epithelial cells incubated in tears collected from a patient affected by keratoconus. Hstopathological analysis of KC corneas shows thinning of the epithelium and the stroma within the apical cone region, Bowman's layer breaks, focal fibrosis, and apoptosis of the anterior stromal keratocytes. Human epithelial cells are challenged with stressful conditions, either a combination of IL6 and TNFalpha, or human tears from moderate keratoconus patients (InViMOS platform), or low-pH conditions.

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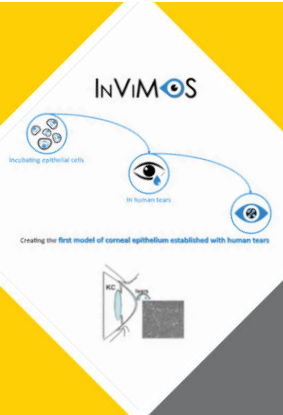

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3. **3D matrix**- In a more complex scenario, given matrix thinning is one of the end results in patients with KC, a 3D models (cells encompassed in a semi permeable hydrogel matrix) will be used to evaluate Human corneal fibroblast / or epithelial cells viability and secretion of specific extracellular matrix (ECM) components.

Readouts

The following parameters will be taken into consideration:

Models 1-2

- **Cell proliferation:** quantitative evaluation of cell proliferation, either in the presence/absence of Client's compounds.
- **Apoptosis:** quantitative evaluation of the apoptotic caspases-3 and -7 either in the presence/absence of Client's compounds.
- **Oxidative stress:** cells will be incubated in oxidative stress-related conditions (low-pH conditions, with or without hydrogen peroxide (H_2O_2)) and reactive oxygen/nitrogen species (ROS/RNS) production will be measured with 2',7'-dichlorodihydrofluorescein diacetate dye
- **Immunomodulators expression:** the expression profiles of immune mediators that play an important local role in the pathology will be examined in cells using real-time PCR and multiplex bead-based immunoassay (IL6, IL1, TNF□, TGF□, IL10)
- **Metalloproteasis expression:** the modulatory effect of Client's compounds on metalloproteasis (MMPs) -1, -2 and -9 and their tissue inhibitors (TIMPs) production by cells will be evaluated by real-time PCR and immunoassay.

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Models 3

Study for matrix secretion and deposition in 3D model: cells will be seeded in polycarbonate transwell plates and stimulated with a stable Vitamin C derivative (0.5 mM 2-O-a-D-glucopyranosyl-L-ascorbic acid) in order to evaluate expression and secretion of specific extracellular matrix (ECM) components. Media will be changed every 2 days for 4 weeks.

The levels of selected markers of interest (for example Collagen I, III, and V, fibronectin, Smooth muscle actin SMA, MMP-1, MMP-3 and TIPMs) or specific markers of stromal components that are altered in KC stroma, will be quantitatively evaluated.