Towards tissue engineering of corneal epithelium

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Tissue engineering of cornea is a regenerative technology with a big potential in cornea transplantation to restore lost vision [1]. Human cornea has at least two types of local stem cells: limbal epithelial stem cells (LESCs) [2] and corneal fibroblast (keratocytes) [3]. For LESCs expansion most of authors still use superficial corneal biopsy [4, 5] and a feeder layer from inactivated mouse 3T3-fibroblast [6, 7]. However, the corneal fibroblasts, which can be cultured in vitro, can probably be used as a feeder layer for LESCs expansion. Our new corneal biopsy protocol is available for collect two types of cells in a single small biopsy sample [8]. Taking into account that the maximal density of keratocytes is located in 10% of stroma surface, we use deep cuts biopsy (120 μm).

Primary human cornea cell culture were obtained by original mechanicaly fixing methods from small pieces of cornea (1-2 mm^2) from limbal zone (procedure approved by the Ural State Medical Academy Local Ethics Committee), and cultured in DMEM (high glucose) medium with 10 % fetal bovine serum. Matrix topography was measured using atomic force microscopy (NT-MDT Ntegra Aura and Life) on air and in liquid. Corneal epithelial progenitors and keratocytes mixed cell culture were seeded on the poly-hyaluronan matrix. We cultured them during 14 days submerged in media using the so-called “air-liquid” method. The harvested matrix samples were fixed in formalin and embedded in paraffin. To obtain preliminary morphological information, the sections were stained with hematoxylin and eosin (H&E) (Fig.1).

Phase contrast microscopy indicated that the primary cells from the limbal explants form a feeder-like fibroblast cells monolayer with colonies of epithelial-like cells. Cells which were cultured submerged in the media yielded 2-4 epithelial cell sheets on the matrix; while those cultured using the “air-liquid” method yielded 3-5 epithelial cell sheets on the matrix, including the basement membrane. AFM measurements on the poly-hyaluronan matrix revealed a structure of interwoven fibres with a typical size ranging from 60 to 130 nm (Fig.2). The structure remained stable in water, however the rms roughness was reduced by half as compared to the dry state, from 68 nm to 31 nm.

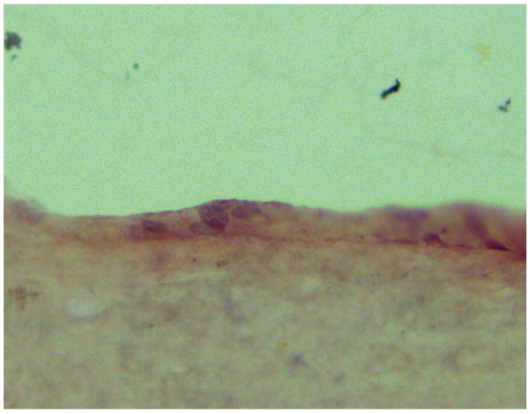

Fig. 1. Cross-section of the cell layer and basal membrane

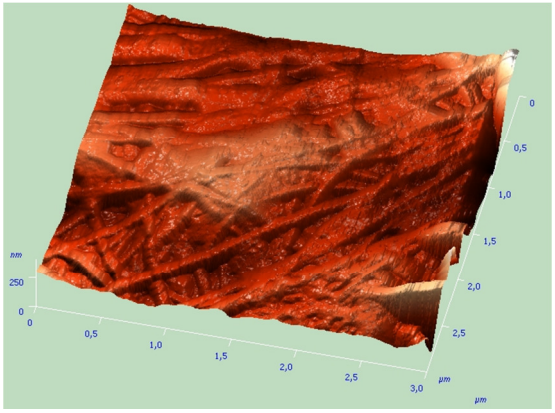

Fig. 2. Morphology of the poly-hyaluronan matrix in dry state.

In conclusion, we demonstrated that the poly-hyaluronan matrix is a suitable candidate for culturing cornea cells. The matrix possesses fine interwoven structure which is retained in aqueous solution. The cells in the cornea mixed cell culture were found to adhere to the poly-hyaluronan matrix. In the future we plan to confirm the presence of LESCs and keratocytes in the culture using specific markers, e.g. p63, CK3, ABCG2 [9], Keratocan and ALDH3A1 [10].

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