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MORPHOLOGICAL STUDY OF THE REGENERATION OF DAMAGED CORNEA STROMA

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ABSTRACT

The purpose of this study was to determine the source of the cells participating in regeneration of corneal stroma. The cornea of experimental animals was perforated up to lens by sterile preparative needle. This process was investigated in white adult mice by means of histological method. For histological investigations material was fixed after 3,6,18 hours and further after every day within 10 days after operation. Moreover, material was obtained 20 and 35 days after operation. Data of the histological studies have shown that 3 hours after injury the stretches began to grow from the inner side of the limb; reaching an injured area after a day. 5 days after injury the wound was entirely covered by epithelial cells. At 30-35th day stroma was completely regenerated.

KEYWORDS: Stroma; corneal limbus; stretches; fibroblast-like cells; myofibroblast; stem cell (SC).

1. INTRODUCTION

Cornea is a transparent, external part of the supporting layer of the eye and thus is often exposed to traumas due to its topographic position on eye front surface. According to the literature regeneration of injured corneal stroma proceeds at the expense of fibroblasts. Those cells are well known and described in details; however, their origin remains an open question and the source of regeneration of injured corneal stroma is an important problem. It is not clear whether new formation of stroma happens at the expense of innate cells or elements of hematogenic nature. The goal of our work is to elucidate this question.

Early[1-2] and recent majority of the works[3-5] deal with regeneration of epithelium of injured cornea and ways of its treatment. These authors mentioned, that the maintenance of a healthy corneal epithelium under both normal and wound healing conditions is achieved by population of stem cells (SC) located in the basal epithelium at the corneoscleral limbus. The characteristics of the specific microenvironment of corneal SC, as provided by growth factor activity and basement membrane heterogeneity in the limbal area, could serve as additional tools for their selective enrichment and **invitro** expansion for the purpose of ocular surface reconstruction.[6] The human corneal epithelium is being continuously renewed. Differentiated epithelial cells originate from limbal stem cells (LSCs) located in the periphery of the cornea, the corneoscleral limbus.[7] The population of limbal epithelial stem cells (LESCs) are responsible for maintaining the epithelium throughout life by providing a constant supply of daughter cells that replenish those constantly lost from the ocular surface during normal wear and tear and following injury. LESCs deficiency leads to corneal opacification, inflammation, vascularization and discomfort.[8-9] These results indicate that scleral fibroblasts have an increased capacity for myofibroblast formation which appears to negatively affect their ability to support LEP (limbalepithelial progenitor cells) growth. Superior growth of LEPs in the presence of limbal fibroblasts indicates a role for limbal fibroblasts in promoting the proliferation of limbal epithelium during the wound healing.[10] This study confirmed that the small molecular compound pluripotin promoted the proliferation of rabbit limbal epithelial cells by improving the expansion of limbal stem/progenitor cells ***in vitro.***[11] These results show that TGF-β (transforming growth factor-β) plays an important role in directing local inflammatory responses in ocular surface epithelial cells.[12] Their findings suggest that vitamin B12 treatment represents a powerful strategy to accelerate not only re-epithelization but also corneal regeneration after mechanical injury.[13] The purpose of this article is to review the factors involved in the maintenance of corneal transparency and to highlight the mechanisms involved in the appearance, persistency and regression of corneal opacity after stromal injury. The development of corneal opacity involves complex processes mediated by cytokines, growth factors, and chemokines and corneal epithelial-stromal interactions that involve the epithelial

basement membrane—that may lead to myofibroblast generation, a decrease in cellular corneal crystallins, and loss of stromal structural components. A better understanding of cells and molecules involved in this process may lead to new treatment options to restore corneal transparency and prevent corneal scar formation.[14] Thus, after epithelial and epithelial BM injury, stromal keratocytes contribute important perlecan and nidogen-2 components to the regenerating epithelial BM.[15] In organotypic cultures, the morphological changes in the CESs and the expression patterns of the growth factors in the stromal cells clearly demonstrated stromal-epithelial cell interactions, and the results suggest that stromal cells and epithelial cells may act in concert in the cornea **[16].** These data support a role of MMP12 in promoting early repair processes following corneal epithelial injury by enhancing epithelial cell migration and neutrophil infiltration.[17]

2. MATERIALS AND METHODS

2.1 Materials

To determine the origin of stroma cells involved in regenerating of the cornea white adult mice by means of histological method.

2.2 Histological method

The cornea of experimental animals was perforated in the centre up to lens by sterile preparative needle. At different time intervals after damage the lens was extracted and the cornea was cut along the limb.

For histological investigations the operated corneas were fixed after 3,6,18 hours and further after day up to 10 days after operation. Moreover, material was obtained 20 and 30 days after surgery. Cornea was fixed in 10% neutral formalin and thereafter embedded in paraffin. Sections were stained with hematoxylin-eozin:[18]

1. Deparaffinization;
2. Xylolie for- 10 min;
3. Xylolie for - 10 min;
4. Alcohol 100 for - 3-4 min;
5. Alcohol 95 for -3-4 min;
6. Alcohol 70 for 3-4 min;
7. Rinsing well in aqva destilata;
8. Leaving in aqva destilata for awhile;
9. Pouring of hematoxine-eozin by pipette on the plate glass and leaving for 7- 10 min;
10. Rinsing well in running water;
11. Rinsing in aqva destilata well;
12. Hematoxylin-eozin for- 2 min;
13. Rinsing in aqva destilata;
14. Alcohol + aceton(one part 100+one part aceton) rinsing very quickly;
15. Alcohol 100 for 3-5min;
16. Xyloliefor 3-5 min;
17. Ebedding in balsam.

3. RESULTS

Data of histological studies

Data of histological studies showed that full-thickness perforating injury of white adult mouse cornea is followed by the exudative period of inflammation, which changes to the proliferative phase, during which dense cellular infiltrates are formed. 3 hours after injury the wound with dissected vertically edges passes across all layers in the central part of cornea. At the same time stretches of cells directed towards wound move off the limb vessels.

24 hours after injury here and there unstructured material accumulates which is intermingled with cells of the damaged corneal area. The above mentioned stretches reach injured area only after a day. The stretches begin to grow from the inner side of the limb, where circular blood vessel is located which is the basis of blood supply of inner parts of an eye (Fig. 1, 2). There is a cavity within the stretches, where stretched cells are arranged in parallel. 5 days after injury the wound is entirely covered by epithelial cells. The epithelium has the typical appearance of multilayer plane epithelium of a cornea. Stroma is not regenerated yet, only a thin layer of a basic material occurs. The rest of the wound cavity is filled with the infiltrate (Fig. 3, 4, 5). At 20th day after injury the corneal stroma is almost recovered (Fig. 6). Later, at 30th day the infiltrate is loosened, stretches are devastated and stroma is entirely regenerated (Fig. 7).

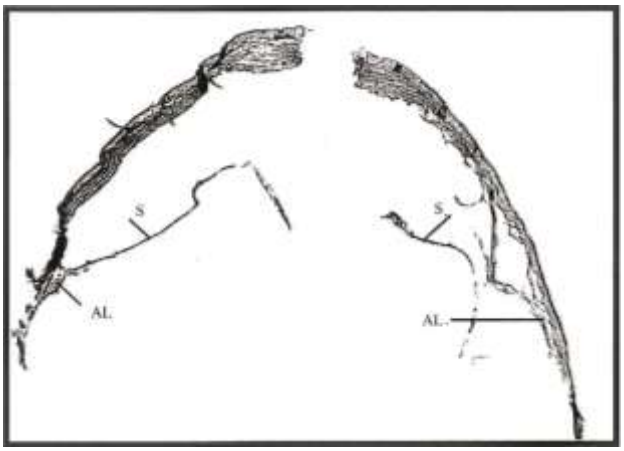

Fig. 1: Cross-section of adult white mice cornea.
18 hours after opera.
Formation of stretches (S) in the limbal area (AL). Fixation 10% formalin; H&E staining; magnification: 2,5X.

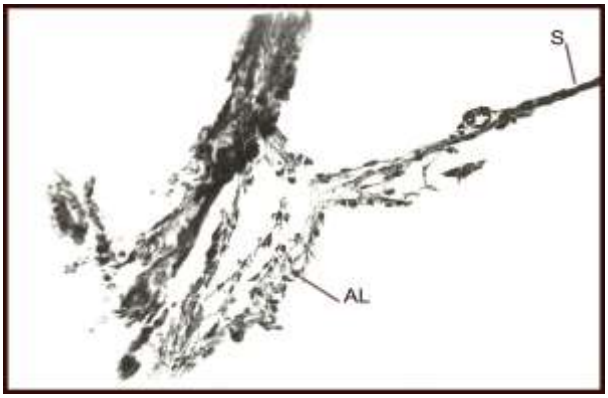

Fig. 2: Cross –section of adult white mice cornea.
18 hours after operation.
Formation of stretches (S) in the limbal area (AL).
Fixation 10% formalin; H&E staining; magnification: 40.

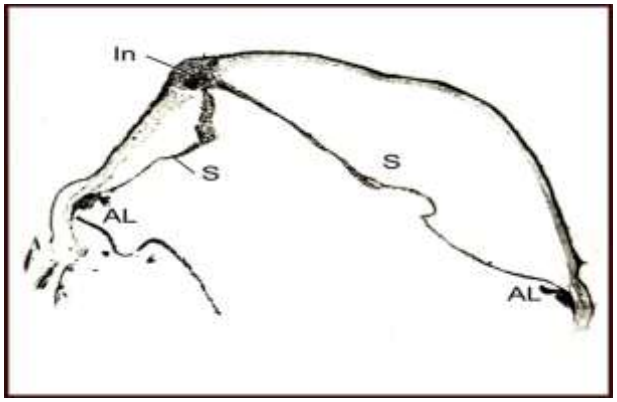

Fig. 3: Cross-section of adult white mice cornea.
5 days after operation.
Formation of a thin layer of basic in 5 days after operation. Cellular Infiltrate and the stretches formed in traumatic cornea. Infiltrate (In) takes up the most part of wound; the stretches (S) go to the infiltrate from the limb area (AL).
Fixation 10% formalin. H&E staining; magnification: 2,5X.

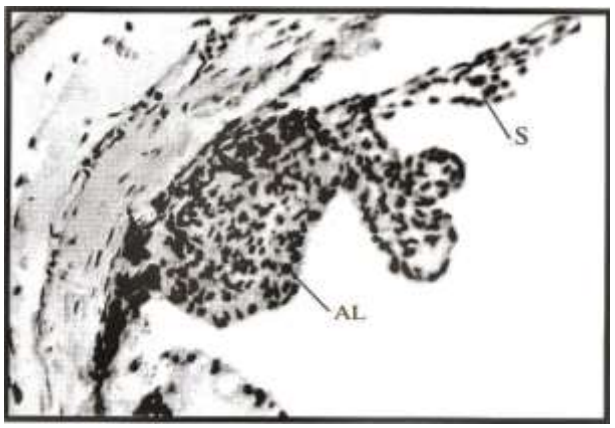

Fig. 4: Cross –section of adult white mice cornea.
5 days after operation.
Limb area (AL), where stretches (S) directly pass to the limb tissues. Left side.
Fixation 10% formalin. H&E staining; magnification: 40X.

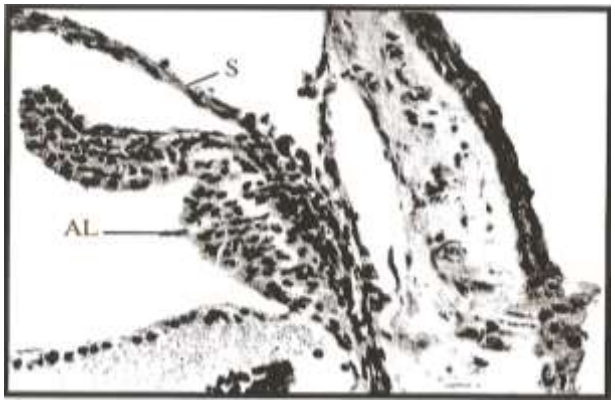

Fig. 5: Cross –section of adult white mice cornea.
5 days after operation.
Limb area (AL), where stretches(S) directly pass to the limb tissues. Right side.
Fixation 10% formalin. H&E staining,; magnification: 40.

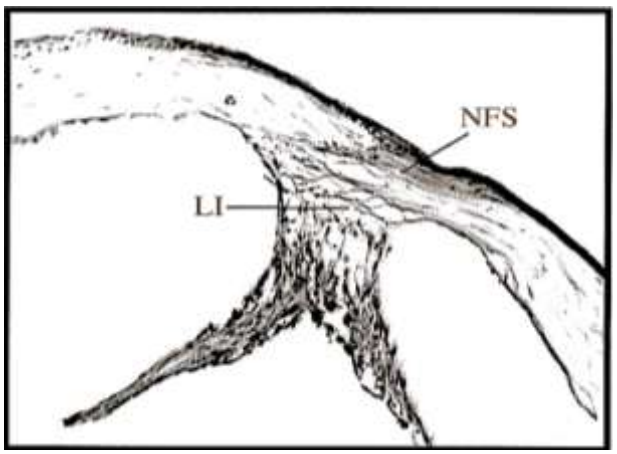

Fig. 6: Cross –section of adult white mice cornea.
20 days after operation.
Newly-formed stroma (NFS) and loosened infiltrate (LI).
Fixation 10% formalin. H&E staining; magnification: 16X.

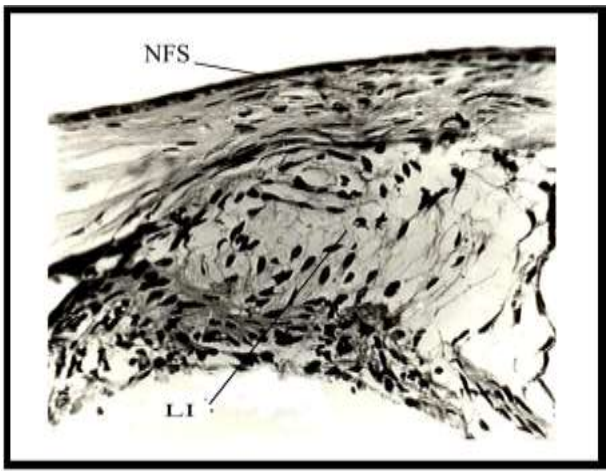

Fig. 7: Cross-section of adult white mice cornea.
30 days after operation.
Newly-formed stroma (NFS) and loosened infiltrate (LI).
Fixation 10% formalin. H&E staining; magnification: 25X.

4. CONCLUSION

Results of histological investigations have shown: new structural elements-stretches extended from eye limb up to the wound infiltrate are likely the main paths of migration of fibroblast-like cells, which take part in regeneration of injured corneal stroma.

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