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Corneal Stromal Remodelling Using Stem Cells-Advances and Potential Application: A Literature Review

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Abstract

Regeneration of the ocular tissues to the healing of the wounds required many complex processes as migration, mitosis, and differentiation of epithelial cells and stromal fibroblast. Currently, corneal transplantation is the most common treatment for any damage to these layers; however, that procedure is associated with a higher risk of transplant rejection and limited by the availability of donor tissue. In order to develop more robust treatments for corneal damage, growing research has been focusing on corneal stem cell. Over the last few years, stem cells have constituted a therapeutic revolution in the regeneration of damaged organs and tissues and restoring both integrity and function. Several types of stem cells have been investigated and applied in experimental models and clinical trials, including bone marrow stem cells, adipose - derived stem cells, endothelial progenitor cells, human dermal fibroblasts, and keratinocytes for wound healing. Corneal stromal stem cells maintain a corneal phenotype through many population doublings, and modulate the host immune response; thus, generating bioengineered stromal constructs in the cornea. Previous research addressed the positive impacts of corneal stromal stem cells on repairing corneal damage, corneal scarring, and blindness, in addition to the utility in bioengineering stromal tissue. In this review, we aimed to address the current progress and clinical applications of corneal stromal stem cells in stromal wound healing.

Keywords: Stem cells; Wound healing; Bioengineering; Corneal stromal remodeling

Introduction

Cornea stromal anatomy

The human corneal layers can be classified into five layers; three cellular layers (epithelium, stroma, and endothelium) and two interface membranes (Descemet membrane and Bowman membrane) (Figure 1) [1,2]. The main bulk of the structural framework of the cornea is provided by corneal stroma that constitutes up to 85% of the thickness of the cornea. In the seventh week of gestation, and due to the neural crest migration, the stroma is formed after the establishment of the primitive endothelium [2-5]. Histologically, it's considered as a cellular collagenous structure; however, it differs from other collagenous structures in many aspects as transparency, the organization of collagen fibrils, and extracellular matrix (ECM) [5].

The corneal stroma is formed of three parts; collagens, proteoglycans, and cells. Moreover, it has specialized glycoproteins and ions that responsible for organizing the collagen fibrils to maintain transparency [2,6]. The collagen fibrils of the stroma are arranged in parallel bundles called fibrils, which are laid down within layers or lamellae, which have a variable thickness (300-500 lamellae); increases peripherally at the limbus and decreases centrally, with higher packing density in the anterior lamella than in the posterior ones [7,8]. These organized fibrils work on reducing forward light scatter and have a significant role in the transparency of the stroma [9].

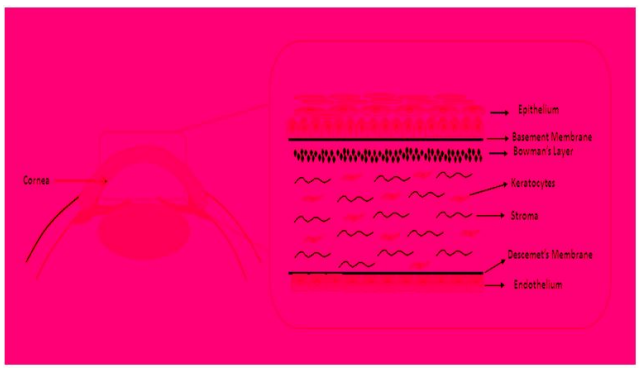

Figure 1: Structure of cornea showing corneal epithelium, stroma, and Descemet's membrane.

It was found that the stromal collagen fibrils are composed of type I and a large amount of type V collagens. Type I collagen is found in a heterodimeric complex with variable diameters, while type V provides a unique and small diameter [10]. These structures are surrounded by a kind of matrix gel contains mucopolysaccharides, keratan sulphate, chondroitin sulphate, and dermatan sulphate. This gel is defined as glycosaminoglycans (GAGs) which are attached to a core protein, they

called the whole molecule a proteoglycan [5,11]. These sulfate groups are very important for the function of proteoglycans; dermatan sulfate binds water at the hydration level, while the keratan sulfate are not and this suggests that the keratan sulfate acts as a reserve for hydration [12,13]. Furthermore, the whole size of the proteoglycans is small enough to fit the spaces between the collagen fibrils [12,14]. The proteoglycan has an important role in the regulation of stromal hydration and corneal transparency. There are four types of core proteins of major proteoglycans in the adult corneal stromal ECM: mimecan, decorin, keratocan and lumican [5]. The core proteins have a similar size ranged between 35-40 kD. In terms of stromal cells, the major cell type is keratocytes that have a significant contribution in maintaining the ECM environment and synthesizing the collagen molecules and GAGs [2]. The corneal stroma performs numerous critical roles within the eye. Optically, it is the main refracting lens and thus has to combine almost perfect transmission of visible light with precise shape, to focus incoming light. Besides, mechanically it has to be very firm to preserve the inner contents of the eye. Its structure at all hierarchical levels governs these functions.

Wound Healing Events and The Role of Growth Factors

Regeneration of the ocular tissues in order to the healing of the wounds required many complex processes as migration, mitosis, and differentiation of epithelial cells and stromal fibroblast. After a few hours of injury, the epithelial cells begin to interact with the ECM and migrate from the edge of a wound [15]. A new layer of stratified squamous epithelial cells is formed from repeated mitosis of the surrounding cells to replace the migrating cells and resurface the defect. These processes are regulated by many peptide growth factors as a Keratinocyte Growth Factor (KGF), Epidermal Growth Factor (EGF), Platelet-Derived Growth Factor (PDGF), Hepatocyte Growth Factor (HGF), and Transforming Growth Factor (TGF)-b [16]. It was observed that the tear film contains some molecules of EGF that can penetrate the injured epithelial layer to stimulate the epithelial cells. This mechanism can occur through an autocrine pathway; the epithelial cells contain EGF mRNA that can be synthesized into EGF to stimulate the healing processes [17,18]. The central corneal cells have very sensitive HGF receptors that rapidly expressed to the released HGF from the fibroblast after the epithelial injury to re-epithelialize the wound. Moreover, HGF works on inducing the motility of the cells through transactivation of the EGF receptor [19]. In human corneal epithelial cells, the binding of HGF to c-Met activates mitogen-activated protein kinase (MAPK) pathways through the receptor-Grb2/Sos complex to the Ras pathway or through protein kinase C (PKC) [20]. Many biological factors are required for the epithelial cell survival as a phosphatidylinositol-3 kinase (PI3K) and p70 S6 kinase (S6K) that are regulated by PKC and protein kinase B [21].

In case of a corneal stromal wound, an increased expression of actin was observed, which lead to differentiate the keratocytes into spindle-shaped fibroblasts (a migratory phenotype) to proliferate and migrate towards the injured area. During this differentiation, some keratocyte proteins such as keratan sulfate proteoglycans and corneal crystallins are down-regulated to remodel the wounded ECM [22,23]. The corneal wound bed is formed as a product of these processes. Furthermore, the fibroblasts differentiate into myofibroblasts, which are characterized by the expression of α-smooth muscle actin (α-SMA) that has a significant role in corneal wound contraction [24]. All of these differentiation, proliferation, and transformation processes of Keratocyte-Fibroblast-Myofibroblast (KFM) are regulated by TGF-β1 and PDGF [25].

Carrington and his colleague investigated the effects of HGF and KGF on early corneal epithelium and stromal wound healing. They reported that the KGF accelerated the epithelial coverage of the wound, while the HGF did the opposite. However, the presence of HGF enhanced the keratocyte repopulation of the denuded area under the wound, while it decreased in response to KGF. Therefore, they recommended inhibiting HGF in case of persistent epithelial defects [26]. Pastor and Calonge conducted an RCT multicenter study to investigate the effect of EGF on corneal wound healing. They randomly assigned 47 patients to topical EGF and 57 patients to placebo [27]. At the end of the trial, they found that the EGF significantly ($p<0.01$) decreased the time of healing in the group of EGF (44.17 h) compared with the placebo group (61.05 h). In contrast, Dellaert et al. showed that there is no significant acceleration of corneal re-epithelialization in the topical EGF group when compared with the placebo group [28]. They explained this by the possible down-regulation of the receptor sites after the keratoplasty. However, this finding was confirmed by Cohen et al. who reported that the topical application of epidermal growth factor onto partial-thickness wounds in human volunteers does not enhance re-epithelialization [29]. Regarding the fibroblast growth factor, Meduri et al. showed that the combination of basic fibroblast growth factor and cysteine was significantly accelerated the corneal re-epithelialization after keratectomy in patients with myopia [30].

General Overview on Stem Cells and Characteristics of Corneal Stromal Stem Cell

Over the last few years, stem cells have constituted a therapeutic revolution in the regeneration of damaged organs and tissues and restoring both integrity and function [31]. Several types of stem cells have been investigated and applied in experimental models and clinical trials, including bone marrow stem cells, adipose-derived stem cells, endothelial progenitor cells, human dermal fibroblasts, and keratinocytes for wound healing [32-34]. Stem cell immunomodulation has been extensively researched for their anti-fibrotic/pro-regenerative effects in wound healing in various organs [35,36]. The cornea is one of these organs. The cornea contains three cell types, the stratified surface epithelium, the stromal keratocytes, and the innermost endothelial cells. Trauma or infecting to these layers may lead to corneal scarring, visual impairments, and blindness. Currently, corneal transplantation is the most common treatment for any damage to these layers; however, that procedure is associated with a higher risk of transplant rejection and limited by the availability of donor tissue. In order to develop more robust treatments for corneal damage, growing research has been focusing on corneal stem cells.

The corneal stem cells reside at the limbus, the border of the cornea and sclera, and involve two cell types; the epithelial and stromal stem cells [37-40]. The epithelial stem cells in the cornea are generally known as the limbal stem cells. Transplantation of limbal cells are commonly used for treating patients with corneal epithelial damage such as for *in situ* epithelial regeneration, or *ex vivo* expanded sheets of replacement cells [41,42]. The corneal stromal cells contain a highly organized extracellular matrix interspersed with keratocytes. The roles of keratocytes involve supporting the corneal epithelium, maintaining the extracellular matrix, and producing collagen lamellae and proteoglycans which in turn keep the transparency of the corneal stroma [8,12,43]. A highly organized corneal extracellular matrix is

essential for light transmission, but this structure may be interrupted during injury or disease, resulting in vision impairment.

The limbal stem cells are commonly used in corneal repair more than corneal stromal stem cells; however, recent evidence has shown that the corneal stromal cells have great therapeutic potential in this area, especially in tissue-engineering approaches [44,45]. Thus, corneal stromal cells are essential in the provision of cells for corneal maintenance and regeneration. Also, corneal stromal cells are able to reorganize a disrupted matrix and restore transparency in scarred corneas [17,46].

In addition to characteristics of adult stem cells, the corneal stromal stem cells have shown another interesting effect when injected into mouse corneal stroma, which did not cause immune-mediated rejection of these cells. A transient inflammatory response, mostly neutrophils, occurred but diminished within a week. A similar transient response with significantly higher CD45+ cells occurred after injection of human corneal fibroblasts within one week. After two weeks, the injection of corneal fibroblasts displayed CD3 T cells; however, there were no T cells in tissue injected with corneal stromal stem cells. Further, the eyes injected with stromal stem cell were clear, while the eyes injected with fibroblast displayed visible haze within two weeks. Finally, after corneal stromal stem cells injection in chimeric mice rescued with green fluorescent protein-bone marrow cells, a transient influx of green cells occurred. Contrary, injection with corneal fibroblasts resulted in a robust influx of green cells into the cornea within ten days. All the previous data prove the immunomodulatory function for corneal stromal stem cells [44].

The Clinical Applications of Corneal Stromal Stem Cells

Corneal stromal stem cells maintain a corneal phenotype through many population doublings, and modulate the host immune response; thus, generating bioengineered stromal constructs in the cornea. These constructs could help in replacing the scarred stroma by partial thickness transplantation. Also, such constructs could provide an alternative substance without complications which decrease the use of cadaveric donors. Evolving of stromal replacement constructs with corneal stromal stem cells will consequently progress the therapeutic applications of these stromal cells. Besides its use in bioengineering stromal cells, corneal stromal stem cells may play a role in producing a direct cell-based treatment for patients with corneal scars [47].

Du et al. reported such a model of cell-based treatment using murine mice with corneal opacity similar to that of scar tissue as a result of disruption of stromal collagen structure. After injection with human stromal stem cells, stromal thickness, collagen fibril defects, and corneal transparency were restored to normal. An interesting finding was that corneal stromal stem cells did not elicit an immune T-cell response [44]. The ability of stromal cells to suppress T-cell rejection may help in avoiding immune rejection of most allogenic tissue transplants. Such a feature of these cells, maintained in vitro, could provide a therapeutic tool for mediating inflammatory response and tissue rejection in transplants or other situations.

Previous research addressed the positive impacts of corneal stromal stem cells on repairing corneal damage, corneal scarring, and blindness, in addition to the utility in bioengineering stromal tissue [48]. Basu et al. assessed the effectiveness of limbal biopsy-derived corneal stromal stem cells on a mouse model with corneal scarring. After engrafting into the corneal wound of the mouse, corneal stromal stem cells prevented the fibrotic formation and induced regeneration of transparent native corneal tissue [49]. In an experimental study by Morgan et al. corneal stromal stem cells significantly increased wound healing after laser in situ keratomileusis-like flap while maintaining corneal transparency. This may be due to the deposition of extracellular connective tissue similar to the normal tissue, and by the reduction of activated keratocytes, which are recognized to scatter a large amount of the incident light [50]. Hertsenberg et al. investigated the mechanism by which corneal stromal stem cells prevent the formation of fibrotic tissue. They found that a neutrophil infiltration played a role in the fibrotic response to the damaged cornea and that corneal stromal stem cells up-regulated the secretion of TSG-6, a protein that regulates neutrophil migration, which prevented scarring [51].

Another study by Mukhey et al. reported that human corneal stem cells have the ability to generate cells and organize extracellular matrix in Real Architecture For 3D Tissues equivalents for transplantation. Thus, they may be a valuable bioengineering method to control cell phenotype simultaneously [52]. A recent study showed that a mixture of engineered silk films and corneal stromal stem cells produced an optically and mechanically functional corneal stromal tissue equivalent in a 3D multi-lamellar structure [53].

Furthermore, it was reported that corneal mesenchymal stromal cells have favorable antiangiogenic effects. Eslani et al. found that corneal mesenchymal stromal cells achieved high levels of antiangiogenic factors and low levels of the angiogenic factor. In vivo, application of corneal mesenchymal stromal cells to injured mouse corneas inhibited the growth of corneal neovascularization. These data point to the direct antiangiogenic roles of corneal mesenchymal stromal cells and their promoting clinical application for preventing pathologic corneal neovascularization [54].

Guo et al. induced corneal stromal cells to form an extracellular matrix by adding a vitamin C derivative. Parallel arrays of fibrils with alternating directions were constructed within the extracellular matrix, and they were similar to the developing mammalian stroma. This model may offer a scaffolding appropriate for tissue engineering a biomimetic stroma [55]. Another study by Carrier et al. used both dermal and corneal fibroblasts and reported that the natural stromal cells could be imitated [56].

Recent Advances and Future Research

Progress in corneal stem cell research offers an optimistic opportunity for their use in regenerative medicine and tissue engineering. Corneal stroma engineering has been actively investigated by developing functional corneal stroma substrates through chemical, morphological, and mechanical cues [57]. There are other approaches for the corneal stromal engineering such as animal sources as an alternative to the native tissue. Given the high risk of immune-mediated rejection, the fully acellular cornea has been recommended to overcome this rejection.

A decellularized animal tissue provides a three-dimensional extracellular matrix, advantageous biocompatibility, sufficient biomechanical tension and high transparency that mimics the native cornea and can be applied with or without the addition of a cellular component [58]. Animal sources such as pigs, cats, and cows are used for grafting [59-63]. Decellularized corneal stroma maintains basement membrane structures; therefore, it was used as a carrier for epithelial transplantation in experimental animals, and as a matrix scaffold for

limbal stem cell expansion *in vitro*, with good outcomes [64,65]. Decellularization could be utilized not only in xenogeneic but also in the allogeneic cornea for transplantation. Theoretically, this would decrease the associated complications and improve clinical results of allogeneic corneal transplants.

Future studies could assess the utility of pluripotent stem cells or other adult stem cells to restore the corneal stroma, with guaranteeing safety measures before transplantation. Cell treatments for each corneal layer will focus on a specific disorder, instead of a full or partial thickness corneal transplant, which is the present therapy. Moreover, one donor cornea will potentially treat multiple patients if the cells are successfully cultivated, and certainly, this would have an optimistic effect on the shortage of donor corneas worldwide. Further studies should be conducted and focused on exploring human stromal stem cells, their immune privilege, and their potential in tissue engineering.

Conclusion

In conclusion, the currently published experimental studies support the beneficial impact of corneal stromal stem cells on repairing corneal damage, corneal scarring, and blindness, in addition to their utility in bioengineering stromal tissue. Future studies could assess the utility of pluripotent stem cells or other adult stem cells to restore the corneal stroma, with guaranteeing safety measures before transplantation. Cell treatments for each corneal layer will focus on a specific disorder, instead of a full or partial thickness corneal transplant, which is the present therapy.

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