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Different Therapies Practiced in Corneal Regeneration

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Abstract

The ocular surface is the outermost part of the visual system where physical or chemical action or injury occurs due to some extrinsic or intrinsic factors like thermal injury, infectious pathogens, burns, Steven Johnson Syndrome or other autoimmune diseases. Some limbal cells have the property to be used as stem cells and carry regenerative property due to their ability to differentiate. Corneal wound regeneration is a complex process where the cell undergoes death, proliferation, migration, differentiation and extracellular matrix remodelling. So corneal regeneration can be done using several forms of therapies out of stem cell therapy is best proven type because of the fast recovery from the infections. Although stem cell therapy is still in earlier stages there are interventions to treat corneal abnormalities is going on. In this process there are several viral and non-viral vectors are used to introduce genes into the cornea *in vivo*, *ex vivo*, *in vitro*. This review focusses on the latest process using biological modulators like gene therapy, signalling inhibitors, micro RNA and nano formulations. This paper also focusses on the latest therapies using stem cells and improvement in ocular drug delivery system to improve the possible and potential therapy of current knowledge for the future treatment of ocular disease.

Keywords: Stem cells; Rock inhibitor; Gene therapy; Corneal regeneration; Micro rNA; Corneal damage

Introduction

As the cornea is the outermost part of the eye it is exposed to many environmental stresses like burns, infections, abrasions and other conditions such as refractive surgeries. In such cases healing of the tissues is triggered. Cornea comprises of 3 types of cells: The stratified surface epithelium, the stromal keratocytes, innermost single layer endothelium cells. The epithelial cells migrate, proliferate and differentiate after the closure of the defect. These are also accompanied by apostates. These keratocytes are replaced by live cells without scarring [1]. Corneal transplantation is done generally but the individual receive LASIK surgeries also contribute 2% complication with abnormal wound healing. Corneal endothelium unlike other cells repairs by cell migration and spreading. Special emphasis is done on the latest process using biological modulators like gene therapy, signalling inhibitors, micro RNA and nano formulations [2]. Corneal wound regeneration is a complex process where the cell undergoes death, proliferation, migration, differentiation and extracellular matrix remodelling. Epithelial renewal is important because it enables the tissue to act as a barrier that protects the interior part of the cornea from being affected by the noxious environmental agents [3-6].

Literature Review

Pathogenesis of corneal damage

Corneal damage is defined as an injury that occurs in the part of the eye which is a transparent covering that helps in imaging of objects known as the cornea about 3% of emergency visits are due to corneal damage. These corneal damages may be mild to moderate and sometimes may also cause vision threatening. Injury of the cornea can be broadly categorized into two that include traumatic (corneal abrasions and foreign exposure) and exposure burns due to chemical, thermal, radiation corneal damage is associated with some diseases related to genetic or degenerative disorders like conjunctivitis, dry eye, keratoconus, pterygium, cataracts, also some infectious agents All the agents cause corneal damage in the following pathway: The corneal epithelium is the softest and smooth part that lacks vascularization and derives nutrients from tear fluid [7]. The severity of burns and infection depends on the duration and intensity of agents that cause damage. When cornea gets exposed to strong alkaline chemicals it causes liquefactive necrosis of cells while acid burns cause coagulation necrosis. Liquefactive necrosis leads to an irreversible injury that contains degenerative neutrophils is more dangerous than coagulation necrosis due to protein denaturation [8].

There are three stages in pathogenesis of corneal damage that include:

Corneal infection: The most common agents include bacteria, fungus, protozoa and parasites. These microorganisms when they enter, they cause a decrease in concentration of host defensive systems mainly on tear film along with some enzymes lysozyme, lactoferrin, phospholipase A2, defensins, statins and cathelicidins which leads to a condition known as microbial keratitis.

Corneal fibrosis: This leads to a loss of transparency of the cornea. Normally the corneal epithelium can renew the cells within 3-4 days of infection by the migration of healthy epithelial cells to the site but when the injury occurs deep into stroma the cells cannot regenerate and leads to serious damage to vision [9]. Epithelial stromal injury in

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the cornea is a starting process in the development of myofibroblasts. These myofibroblasts are produced from keratocytes derived precursors or bone marrow derived precursors play the main role in stromal opacity. From studies, it is clear that TGF-β upgrades the development of mature myofibroblasts also it suppresses IL-1 mediated apoptosis of mature myofibroblasts. Due to this cornea losses its transparency [10].

Corneal neovascularisation: This is a threatening condition caused by several agents. This occurs by IL-8 angiogenic factors. About 1.4 million people per year if left untreated condition may cause edema, lipid deposition and persistent inflammation. Mainly Herpes simplex keratitis leads in developing this condition mediated by Vascular Endothelial Growth Factor (VEGF. The mechanism involved in angiogenesis is increased release of VEGF due to inhibition of VEGF Receptor synthesis (sVEGFR-1 that leads to fragile blood vessels in the cornea. IL-6 and IL-7 also stimulate the production of VEGF. This harms light diffraction mechanisms and leads to blindness when untreated [11].

Gene therapy

Here in this therapy genes are used in treating the disease instead of drugs and surgery. In gene therapy, the mutated gene that causes the disease is replaced with a healthy gene or inactivating (knocking out the mutated gene or introducing the new gene that helps in fighting the diseases can be given to patients [12]. In ophthalmology, the cornea is ideal tissue for gene therapy due to transparent nature and nonvascularized it makes it easy for treating with gene therapy. Although this therapy is still in earlier stages there are interventions to treat corneal abnormalities is going on. In this process there are several viral and non-viral vectors are used to introduce genes into cornea *in vivo*, *ex vivo*, *in vitro*.

In this therapy the genes are incorporated with the help of gene gun, electroporation, intrastromal injection and iontophoresis are used for both viral and non-viral vectors [13].

ROCK inhibitors

Rho kinases (ROCKs are effectors in the Rho pathway which are serine/tyrosine kinases. The main role of ROCKs involved in cell growth, movement, actin cytoskeleton reorganizations that in turn lead to the death of tissue cells. These ROCKs are expressed in the cornea, involve in corneal healing and cell differentiation. So, the use of ROCK inhibitors will improve corneal wound healing. Corneal Endothelial Cells (CEC is the sites of ROCK expression. These cells play a main role in corneal transparency. When ROCK inhibitors are used these cells become inactive and help in processing endothelial regeneration.

The main use of ROCK inhibitors can be seen in several conditions where the outcome is increased retinal blood flow and improved vision. The lists of diseases where ROCK inhibitors are used are listed below along with outcome:

- Fuchs Endothelial Corneal Dystrophy (FECD)-Preserving corneal clarity.
- Failure of central Descemetorhexis-Salvage treatment.
- Glaucoma Increased Intraocular Pressure (IOP).
- Diabetic retinopathy-vasodilation in the retina (optic nerve head) [14].

Ripasudil: It is used in glaucoma treatment with chemical formula $C_{15}H_{18}FN_3O_2S$ known as fluoro-5(((2S)-2-methyl-1,4-diazepam-1-yl) sulfonyl) isoquinoline. It is available in the form of drops and onset of action within 2 hours. The common side effect is conjunctival hyperaemia.

Netarsudil: This is ROCK inhibitors along with norepinephrine transport inhibitory activity with chemical formula $C_{28}H_{27}N_3O_3$ known as (4-((1S)-1-(Aminomethyl)-2-(isoquinoline-6-arylamino)-2-oxoethyl) phenyl) methyl 2,4-dimethylbenzoate. It is also the same onset of action *i.e.* 2 hours after installation of drops and similar side effects as that of Ripasudil [15].

Micro RNAs

MicroRNAs (MiRNAs) are small, non-coding RNA molecules found in multicellular eukaryotic organisms that are important in regulating translational repression of cells [16]. These bind with target mRNAs at 3'-untranslated region for regulating post-transcriptional gene expression. About 25% of miRNAs are found in the corner of the eye and control corneal development, differentiation, glycogen metabolism, post injury regeneration and also maintain Corneal Epithelial Progenitor Cell (CEPC) homeostasis. Whereas in pathological conditions they also regulate keratoconus, corneal neovascularization occurred due to corneal transplantation, herpes simplex virus infection, alkali burns. So, mRNAs became therapeutic targets for corneal diseases [17]. They identified 37 miRNAs (9 in the limbal epithelium, 1 in central corneal epithelium) in the human corneal epithelium. Also, miR-143 and 145, miR-10b, 126 and 155 are located in limbal basal layers of the epithelium [18].

The emerging evidence suggests that miRNAs play a key role in promoting many phases of corneal epithelial wound healing also inhibiting it. During corneal epithelial cell migration and proliferation, miR-205 promotes corneal healing by targeting SH2-containing phosphoinositide-5-phosphatase (SHIP2) which in turn affects Akt signalling pathway for migration of cells and increases motility by modifying F-actin organization. It also inhibits another gene KCNJ10 channel for promoting epithelial cell proliferation. This therapy can either increase natural miRNAs or it may mimic natural miRNAs or inhibit antagomirs when overexpressed [19].

Nano formulation

In nanomedicine, it includes medical application of nanotechnology, nano devices (contact lens) and nanoparticles (silicate, gold, silver, platinum, calcium phosphate, etc.), nanomaterial (nanofibers), nano delivery (liposome, dendrimers, polymeric micelles, nano emulsion) in tissue repair and drug delivery for corneal treatment. The size of the material used in nanomedicine is 10 nm-100 nm. In corneal regeneration nanomedicine mainly focusses on imaging, preventing or reducing corneal opacity and neovascularization.

Nanoparticles include platinum nanoparticles that have anti-aging properties; polymeric nanoparticles made of polyethyleneimine, albumin, chitosan and polyethylene glycol delivers transgene into corneal endothelial *in vitro*; metallic nanoparticles with polymeric nanoparticles like 2kDa PEI with PEI2-Au-NPs deliver genes into the cornea *in vitro*; non-metallic nanoparticles like CaP-NPs (Calcium Phosphate Nanoparticles) is biocompatible and biodegradable that when incorporated into eye dissociate into calcium and phosphate in

corneal endothelial cells and make the cells to regain transparency and clear.

Nanofibers are used in corneal regeneration when the scaffold likes tissue bridging nanostructures that contain peptides. When these are incorporated into cornea the structure of scaffold provides space for cell adhesion and migration for improving corneal repair. Also, octopamine dendrimers coupled with polypropylene imine are cross-linked by collagen are used for corneal cell growth and adhesion.

Nano devices are mainly used for sustained drug release in ocular surgeries. Some example of this is nanospheres made by pullulan and polycaprolactone which include ciprofloxacin coating are mainly used to treat infection of *Staphylococcus aureus* and *Pseudomonas aeruginosa* [20].

Different therapies of corneal regeneration

With corneal epithelium: The corneal epithelium cells *in vivo* healing is slow because it conserves the cell proliferative potential and decreases DNA replication errors. This process occurs in 3 distinct manners:

- The hemi desmosomes which are normally attached to the epithelium matrix and other anchoring structures are lost and a provisional structure compound is formed known as focal contacts. The epithelial cells get flattened and migrate as a sheet and independent of cellular proliferation.
- The cell stratification and differentiation take place.
- Lastly the hemi desmosomes are reformed and extracellular matrix synthesis takes place.

This wound healing process is brought about by complex cascade events including cytokine mediated interactions between the epithelial cells keratocytes of the corneal stroma, corneal nerve, lacrimal glands and cells of the immune system. The level of the interaction is dependent on the inciting injury [21].

With compressed collagen gel: It is one of the most successfully used modulators in tissue engineering because of its high biocompatibility, biodegradability and low antigenicity. Chemical crosslinking shows various advantageous properties when used in implants to replace corneal tissue. Due to its low antigenicity Brown and others developed a technique to produce plastic Compressed Collagen Gel (CCG). These plastic CCG have greatly increased strength, stiffness and can be produced without cytotoxicity to embedded cells. CCG is used as a feeder layer for corneal epithelial cells. It is used as a vehicle that is used to increase the potency of the treatment to millions of individuals with visual impairment.

Extraction of CCG: Compressed collagen hydrogels were made using the RAFT reagents. Briefly, acid soluble rat tail collagen was neutralized and diluted to a concentration of 1.6 mg/ml in a solution containing 1 × minimal essential medium. The collagen solution (typically 1 ml was transferred to 1 wells of a 24 well culture dish containing 12 mm round glass coverslips. The collagen was gelled in a 37°C CO_2 incubator for 1 hour then dehydrated for 15 minutes at room temperature in a laminar flow hood using fibrous absorbers. The collagen gels were transferred to a 60 mm petri dish in sterile Phosphate Buffered Saline (PBS. A sterile disposable biopsy punch with a plunger was used to punch out 2 mm diameter disks to be used for further analysis. CCG gels without cells were stored in sterile PBS. CCG containing cells were maintained in stem cell growth medium in a 37°C CO_2 incubator for up to 2 weeks. After gelation, some CCG gels (without cells were stained with Daylight 633 before sectioning. Gels were rinsed with 0.1 M $NaHCO_3$ and stained with Daylight 633 NHS Ester reactive dye 0.25 mg/ml in 0.1 M $NaHCO_3$, pH 8.5, for 2 hours at room temperature. CCGs were washed in sterile PBS and stored at 4°C until use.

Placement of CCG on cornea: It resulted in firm adhesion of the compressed collagen to the surface of the eye. It is a rapid and convenient method to prevent scarring and help in the regeneration of the corneal tissue. It is beneficial to those individuals who are suffering from corneal scarring and have no alternative therapy.

With umbilical cord: The corneal endothelium consists of a monolayer of cells derived from the neural crest and mesoderm. Its main function is to prevent corneal edema formed by Zonular Occludens-1 (ZO-1) and Na, K-ATPase pump function. The Human umbilical cord is a rich source of mesenchymal cells that acts as an allogenic source.

After inducing differentiation with medium containing Glycogen Synthase Kinase (GSK)-3-beta inhibitors. UC-MSCs formed polygonal structured cells. Expression of major corneal workers was confirmed by Reverse Transcription-Polymerase Chain Reaction (RT-PCR) and quantitative RT-PCR (qRT-PCR).

Western blotting is done to confirm the expression of Na-K ATPase and PITX2. The Localization of Na-K ATPase and ZO-1 occurs in cell junction which indicates the presence of tight junction. So UTECE may be used as an important source of allogeneic cells for the treatment of corneal disease.

Discussion

An open label, single centered the interventional study that is in phase 2 presently started on Feb 9, 2015, for determining the safety and feasibility of cultivated autologous limbal epithelial cells transplantation in treating limbal stem cell deficiency in about 17 patients. The procedure of interventional arm include cultivated autologous limbal epithelial cell therapy is done using a bio-engineered composite of *ex vivo* expanded autologous corneal epithelial cells and along with it they also used an amniotic membrane like Amino graft, Bio tissue that are approved by FDA for reconstructing the ocular surface. A biopsy of 2 mm-3 mm serves as a source of epithelial cells that are expanded on the amniotic membrane in culture and then surgically transplanted onto cornea after excision of fibro vascular pannus. Whereas the procedure of the study arm includes corneal biopsy in the non-diseased eye that provides cells for CALEAC graft. Then the two arms are observed for a time frame of 2 years for outcome of the safety of occurrence of ocular infection, perforation, graft detachment, adverse effects and feasibility of obtaining cell growth and maintaining cell viability also avoiding culture contamination for about 2 years.

A randomized parallel assignment that is quadruply masked in corneal dystrophy, epithelial basement membrane; epithelial recurrent erosion dystrophy; corneal erosions for testing the efficacy of CACICOL20® (RGTA OTR 4120) in improving wound healing and nerve regeneration in anterior cornea in about 40 subjects. Here the subjects undergone therapeutic laser treatment of cornea in single clinic, followed by the installation of treatment or placebo in form of 3 eye drops total (once 2 days after surgery and final time 4 days after surgery). Postoperative eye examinations for measurement of various eye and corneal wound healing parameters are conducted on days 2

and 7 at months 6 and 12. The interventional arm includes an investigational device, regenerating agent, single use doses and topical eye drops that are indicated for corneal wound healing and other group using a placebo with identical packaging and include dosage and administration route. The experimental group includes Cacicol 20 eye drops after laser corneal surgery. 3 eye drops in total that are to be given immediately after surgery. The primary outcome measures the percentage recovery in sub basal nerve density for a time frame of 12 months.

A phase 1/phase 2 study evaluating the safety and efficacy of investigational new drug TTHX1114 (NM141) on regeneration of corneal endothelial cells in patients with corneal endothelial dystrophies following intracameral delivery. This is a multicentre, randomized, masked, vehicle controlled, dose escalation study that includes a non-interventional observational sub-study including 25-50 subjects. The interventional arms include drug TTHX1114 (NM141) engineered FGF-1 delivered intracamerally and other groups include a placebo. While the study arm includes placebo comparator vehicle placebo weekly 4; experimental group: Low dose of TTHX1114 (NM141) weekly 4 and another group mid-dose of TTHX1114 (NM141) weekly 4 also other group high dose TTHX1114 (NM141) weekly 4. The primary outcome changes in corneal endothelial cell count for the time frame of 56 days.

Conclusion

The cornea is the most sensitive part of the eye and is mainly exposed to infections and other forms of damage. So corneal regeneration can be done using several forms of therapies out of stem cell therapy is the best proven type because of the fast recovery from the infections. The stem cell therapy mainly focusses on delivering adult autologous stem cells that are extracted from one's bone marrow or adipose tissue for healing and regeneration of a damaged area of the eye. There is no need for any donor or any other complications with stem cell therapy. But still, there are more and more researches going on in this field for improvement in terms of treatment strategy and eye transplants. Not everyone is going to get an effective treatment using stem cells. When there is severe damage to cornea it cannot be healed easily. Patients should have some undamaged limbal stem cells remaining in one of their eyes for extraction and injection into the damaged eye. In some conditions when some patients are suffering from both cornea infections the stem cell therapy is not easy.

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