Biomaterials for corneal repair and regeneration

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Abstract

Cornea is one of the most vital entities with the major task of bolstering the refractive power of the eye. However it is highly prone to damage and wounds because of various physiological and operative reasons. Thus attempts have been made to accelerate the healing process of the wounded cornea. In this review paper, various mechanisms and systems have been put forth that shed some light on corneal regeneration and tissue engineering. Scaling from the conventional methods of transplantation to the biomimetic activity of various scaffolds (hydrogels, films etc.), alternative techniques have been discussed to counter the problems encountered during keratoprostheses and keratoplasty. With the introduction of biomaterials to corneal healing, tissue engineering has reached its next level of sustainability and efficiency. The combination of cells and biomaterials shows a promising future with regards to the potential for treating damaged corneas.

Keywords*:* corneal regeneration, tissue engineering, biomimetic, scaffolds, keratoprostheses, biomaterials.

1. INTRODUCTION

Worldwide, blindness is caused due to diseases affecting cornea. Corneal damage can be due to mechanical, chemical, autoimmune or biological reasons leading to loss of corneal transparency which ultimately culminates into vision loss (Builles, Janin-Manificat et al. 2010). Causes of corneal blindness include trachoma, ocular trauma, corneal ulceration, and childhood diseases such as xeropthalmia, opthalmia neonatorum and a few viral infections such as *Herpes simplex* viral infections (Whitcher, Srinivasan et al. 2001). The first stage of treatment involves the use of antibiotics and further damage is treated conventionally by corneal transplantation using donor tissue (keratoplasty). But the demand for cornea exceeds the supply and also there exists a chance of immune rejection and disease transmission (Liu, Merrett et al. 2008). Owing to these problems, artificial substitutes for corneal transplantation have been explored. Attempts have been made for reconstruction of cornea by tissue engineering (Builles, Janin-Manificat et al. 2010). The tissue engineering techniques act at a primary level by stimulating local tissue repair, or they can be used at a higher level to replace corneal layers. The general approach involves employing biomaterials as scaffolds to support and direct tissue formation. These systems can be seeded with stem cells, growth factors or be modified with directive proteins. They can either be used to generate new tissue in vitro followed by insertion in vivo or they can be directly used in vivo to repair the damage (Elisseeff, Madrid et al. 2013).

1.1 CORNEA

The cornea is the outermost layer of the eye. It is clear; dome shaped and covers the front of the eye. It is a transparent avascular connective tissue.

1.1.1 STRUCTURE OF CORNEA

The human cornea consists of five layers as shown in Fig.1:

1. ***Epithelium***

   It is the primary protective barrier of the eye made up of squamous, non-keratinized epithelial cells. It forms the tear film-cornea interface which is critical for the refractive power of the eye. Epithelial stem cells serve as a source of new corneal epithelium localized in the limbal basal epithelium (DelMonte and Kim 2011). The epithelium acquires its metabolic supply from aqueous humor and if there is a block in this transport it can lead to epithelium and stromal melting (Ruberti, Roy et al. 2011).

2. ***Bowman's Layer***

   It is the acellular condensate of the anterior portion of the stroma. An injury to this layer leads to formation of scars as the layer is not regenerated.

3. ***Stroma***

   Corneal stroma consists of 80-85% of the corneal thickness. It consists primarily of water (78 percent) and collagen (16 percent), and does not contain any blood vessels. Collagen gives the cornea its strength, elasticity, and form. The stroma is transparent and this is a result of precise arrangement of stromal fibres and extracellular matrix (ECM) (DelMonte and Kim 2011). It is about 500 micrometre thick and consists of collagen fibrils (type 1 collagen) packed in parallely arranged lamellae (Builles, Janin-Manificat et al. 2010). Keratocytes are the major cells of stroma involved in maintaining ECM environment and stromal homeostasis.

4. ***Descement's membrane***

   It is made by the endothelial cells that lie below.

5. ***Endothelium***

   Innermost layer; responsible for maintaining corneal clarity (DelMonte and Kim 2011). Corneal endothelial cell do not regenerate readily.

1.1.2. FUNCTION

Cornea transmits and focuses light; acts as a protective barrier against the outside environment and resists intraocular pressure (Builles, Janin-Manificat et al. 2010).

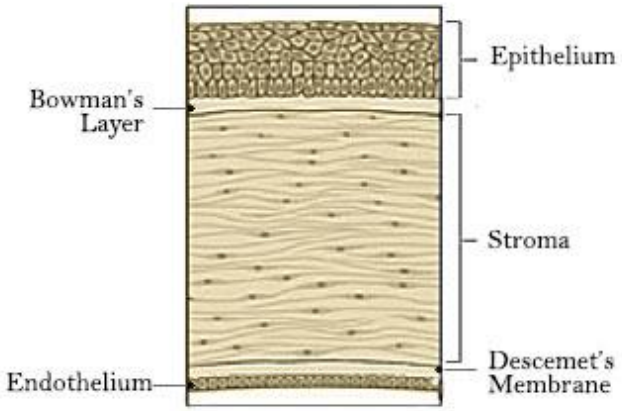

Fig 1. Structure of Cornea

2. KERATOPROSTHESES – A CONVENTONAL TREATMENT FOR CORNEAL HEALING

Keratoprostheses (KPro) is a surgical procedure where a diseased cornea is replaced with an artificial one (device). Keratoprostheses is an alternative to penetrating keratoplasty, with research starting from 1796.

2.1 DESIRED PROPERTIES FOR AN IDEAL KERATOPROSTHESES

Keratoprostheses, or for that matter any implant, natural or synthetic, should have a set of desired properties such as allowing epithelisation to occur, biocompatibility with the host cornea and a high permeability to nutrients and gases (Lai and Hsiue

2007). As keratoprostheses have to be fixed firmly to the cornea, they should be flexible, and not provoke intraocular inflammation or retro-prosthetic membrane formation. Most importantly they should not hinder the normal wound healing process.

But even the best keratoprostheses possess the risk of causing infection, inflammation and being extrusion from the cornea. Attempts have been made to overcome these problems (Hicks, Fitton et al. 1997).

2.2 COMMON KERATOPROSTHESES MODELS

The most familiar models include Dohlman, Strampelli and Cardona.

The Dohlman KPro – It was made with poly (methyl methacrylate) (PMMA) and was a one-stage perforating KPro. PMMA is a solid and rigid optical material. Ulceration and melting of surrounding cornea were the major problems; the ulceration was imputed to the proteolytic enzymes generated by the epithelium and did not occur when cyanoacrylate glues were used to prevent the epithelium or tears from getting under the anterior plate (Dohlman and Doane 1994).

The Strampelli KPro - Corneal healing was found to be improved after incorporation of autologous tissue such as tooth, bone or cartilage in the KPro. Tooth was selected and an osteo-odonto-keratoprostheses (OOKP) was developed. The surgery involved grave complexity, however, a higher retention time was observed. The major post-operative complication was glaucoma.

The Cardona KPro - They were a series of intra-lamellar, penetrating and perforating models. Modifications to this model led to the development of core and skirt KPro by Girard et al. Retro-prosthetic membrane formation and glaucoma were the main complications (Hicks, Fitton et al. 1997).

2.3 ADVANCES IN KERATOPROSTHESES

Recent research for KPros has been focused towards new skirt materials as well as to improve the core-skirt properties (Hicks, Fitton et al. 1997).

Poly(2-hydroxyethyl methacrylate) (PHEMA) and copolymers of HEMA are widely used as biomaterials which include production of soft contact lenses (Chirila 2001). Chirila TV et al have used PHEMA to make one-piece core and skirt KPro. PHEMA is flexible and forms homogenous hydrated gels which have the same refractive index as that of the corneal stroma (Patel, Marshall et al. 1995). But calcium deposition, cell ingrowths and vascularization were notable problems in addition to the reduced mechanical strength (Chirila 2001).

Functional biomedical polymers have been exploited as an advance in the field of keratoprostheses (Lai and Hsiue 2007). Non-Epithelisation of the anterior surface of KPros has been a persistent problem (Hicks, Fitton et al. 1997). Attempts have been made to increase KPro biocompatibility. One of the avenue explored is the development of silicone rubber [SR] membranes grafted with functional groups (Fig.2) which increase the bioactivity (Lai and Hsiue 2007). SR is hydrophobic and an elastic material providing an adequate surface for cell attachment and growth.

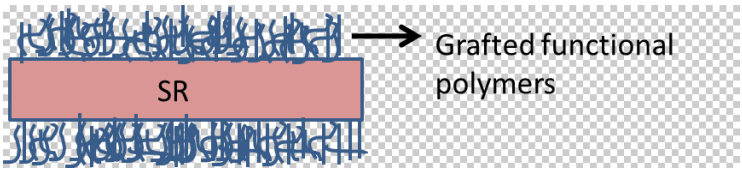

Fig 2. Functional Groups grafted on the SR

Grafting of functional groups has been done through plasma induced polymerization. Further modifications include a homo-bi-functional membrane made of PHEMA-g-SR-g-PHEMA. Hetero-bi-functional membranes have also been made by grafting different functional polymers such as poly (2-methacryloyloxyethyl), acrylic acid and collagen (type I) (Lai and Hsiue 2007).

3. EPITHELIAL CELL SHEET ENGINEERING

Limbal stem cells are a source of generating the epithelial layer of cornea (Elisseeff, Madrid et al. 2013). A penetrating keratoplasty (Fig.3) is done for patients who suffer from limbal stem cell deficiency, but the lack of recipient stem cells does not allow for sustained regeneration of corneal epithelium (Deshpande, Ramachandran et al. 2013). Autologous corneal epithelium has found its clinical application to treat people with corneal-limbal epithelial defects (Lai and Hsiue 2007). Cultured cells cannot be directly placed in the eye, as they won't survive post removal of scar tissue; hence it is preferred to transplant cells which are grown on a substrate. The substrate may either be biodegradable or non- degradable but has to be biocompatible thereby causing minimal inflammatory and immunological response (Deshpande, Ramachandran et al. 2013).

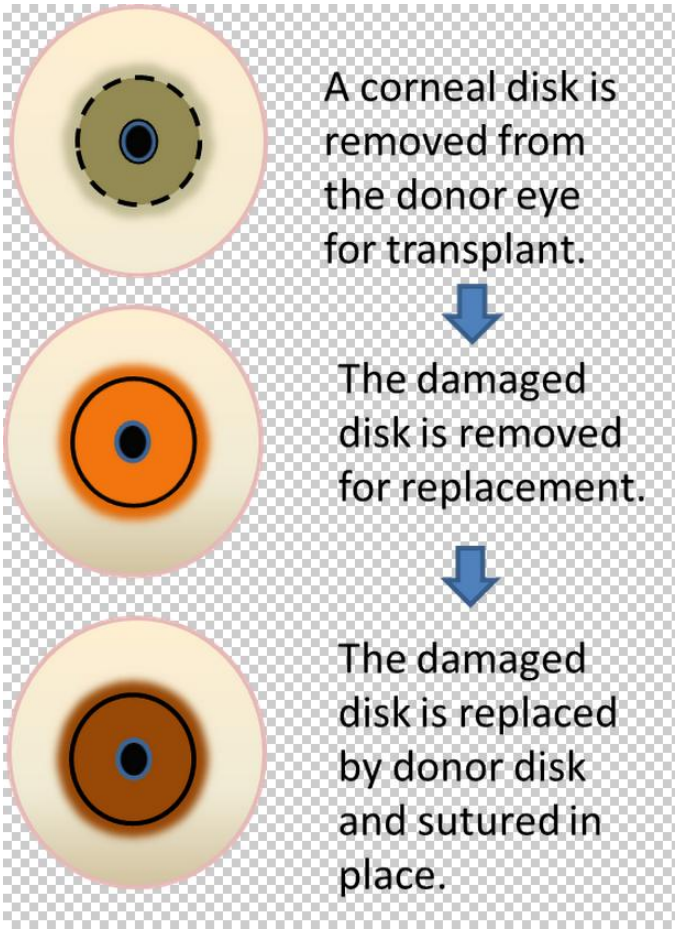

Fig 3. Procedure for Penetrating Keratoplasty

3.1 AMNIOTIC MEMBRANE- A NATURAL SUBSTRATE

Amniotic membrane (AM) has been used as a matrix to carry and expand corneal epithelial stem cells, ex vivo, and act as a bioengineered ocular surface (Lai and Hsiue 2007). The AM acts as graft over which epithelisation occurs. AM not only provides a surface for adhesion and growth of cells but also contains an array of growth factor, anti–vasculogenic factors and anti-inflammatory cytokine (Said, Nubile et al. 2009). The limitation is that autologous cells have to be removed from a healthy contralateral eye. But since AM is a natural surface and only autologous cells are transplanted, prolonged immunosuppression is not required (Tsai, Li et al. 2000). Moreover the outcomes observed under such procedures (especially during the limbal stem cell deficiency treatment) are unpredictable owing to the structural heterogeneity of AM scaffolds. This has been one of the reason why biodegradable hydrogels and other alternatives have been invented to replace the conventional AM based healing (Wright, Mi et al. 2013).

3.2 Poly (D,L-lactide-co-glycolide) - A SYNTHETIC SUBSTRATE

Synthetic biodegradable membranes can be used instead of human amniotic membrane for delivery of cultured limbal epithelial cells. Poly ($_{D,L}$-lactide-co-glycolide) (PLGA) has been studied for its use as a potential substrate owing to its biodegradability and conformity to FDA standards. PLGA is shown to support growth of human limbal epithelial cells but the presence of a feeder layer was essential. The morphology of the cells is found to be the same as on their growth on amniotic membrane. ECM components such as fibrin, laminin or collagen can be added to the membrane to enhance cell growth; best results were found with membranes coated with fibrin. Predictable rates of breakdown can be achieved by varying the ratio of PLA to PGA. PLGA substrate offers a wider accessibility as

compared to amniotic membrane, proving to be a good alternative (Deshpande, Ramachandran et al. 2013).

3.3 SUBSTRATE FREE CELL SHEETS

Tissue engineering methods have been successful to produce corneal cell sheets which can be directly transplanted without the need of a substrate. Also when cell sheets are directly transplanted they adhere to the corneal stroma without the need of sutures. Cell sheets are usually harvested by enzymes such as dispase. These enzymes degrade cell adhesion molecules to detach the cultured cells. But at the same time, cell-cell junction proteins and receptor proteins expressed on the cell membrane are often damaged. Thereby it is recommended to harvest such cultured cell sheets whose cell-cell junctions are less susceptible to such enzymes (Yamato and Okano 2004). To overcome this problem and to produce cell sheets that do not require carrier substrate, dishes coated with temperature responsive polymers have been studied. To produce cell sheets which are less fragile and at the same time do not require carrier substrate, dishes coated with temperature responsive polymers have been studied. Harvesting of cells is done at low temperatures (Yang, Yamato et al. 2005). Oral mucosal epithelial cells have been cultured on temperature responsive culture surface in the presence of feeder cells. The temperature responsive polymer used is poly (N-isopropylacrylamide) (PIPAAm). It facilitates cell adhesion, spreading and growth at 37^{o}C. PIPAAm has a lower critical solution temperature of 32^{o}C. When the temperature is reduced below the lower critical solution temperature, the surface hydrates and swells allowing complete detachment of adherent cells without the need of proteolytic enzyme treatment (Hayashida, Nishida et al. 2005). Kohji Nishida et al have shown that autologous oral mucosal epithelium can serve as an effective alternative to allografts. Cultured oral mucosal are found to be comparable to cultured limbal epithelial cells. The transplanted corneal surface was found to be smooth and clear and no vascularization was observed (Nishida, Yamato et al. 2004).

4. HYDROGELS FOR CORNEAL WOUND HEALING

Hydrogels have been extensively employed as a drug delivery system owing to their ease of manufacturing and self-application (Lee, Singla et al. 2001). They allow diffusion of oxygen and nutrients, have adjustable mechanical properties and are highly biocompatible (Elisseeff, Madrid et al. 2013). A mélange of combinations of polymers were made into hydrogel formulations to study their potential use and applicability. Several hydrogel systems such as that of collagen, gelatin, chitosan, alginate and others have been developed as a substrate for corneal regeneration. They show promising implications for their future use as a vital alternative for keratoprostheses and corneal transplantation.

4.1 COLLAGEN BASED HYDROGELS

Collagen is considered to be one of the most inexpensive and widely abundant material, rendering its use in a myriad of biomedical applications. Collagen poses the required desirable properties like biodegradability, non-antigenicity, biocompatibility, non-toxicity and allows cell proliferation and differentiation (Lee, Singla et al. 2001). Collagen fibrils are the major component of corneal stroma and thus the use of collagen corneal regeneration is justified (Liu, Merrett et al. 2008).

Animal extracted collagen is abundant and inexpensive but high purification is not possible and there is a risk of possible immune response as well as transmission of infectious agents. To overcome these, recombinant human collagen (RHC) has been obtained from transfected mammalian cells, E.coli, yeast cells etc. Recombinant Human Collagen is chemically defined and safe. Its properties and degree of stability is similar to human collagen (Liu, Merrett et al. 2008).

Collagen scaffolds have been used to study cell proliferation and differentiation along with the in vitro modeling of three-dimensional tissues. Researchers have suggested that collagen hydrogels prove to be a convenient scaffold while trying to access the cell membranes as in electrophysiological processes (Ahearne, Liu et al. 2010, Parenteau-Bareil, Gauvin et al. 2010). These hydrogels replicate the conditions of the natural ECM favoring corneal healing by exhibiting phenotypic expressions to injuries. However, it has been observed that the collagen hydrogels have inherently weak architecture and thus are crosslinked to increase its gel strength (Tsai, Hsu et al. 2014). This crosslinking also served to avoid the aggregation of the collagen fibrils at neutral pH thereby controlling the transparency and fibril thickness. Cross linkers, which do not get incorporated in the final product after crosslinking, are used reducing the in vivo toxicity caused by the breakdown of cross linkers (Liu, Merrett et al. 2008). A number of hydrogels have been devised and studied for their optical properties, mechanical properties, equilibrium water content, nutrient diffusivity (especially glucose and albumin), in vitro biodegradation and biocompatibility and UV stability under irradiation so as to mimic the corneal physiology and bio-functionality.

4.1.1. CROSSLINKED COLLAGEN HYDROGELS

1. As previously highlighted, collagen hydrogels are crosslinked in order to improve its gel strength. The most common crosslinking system extensively employed is 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and N-hydroxysuccinimide (NHS). EDC and other water soluble carbodiimides offer a safe and an effective method of stabilization of collagen (van Wachem, Plantinga et al. 2001). The crosslinking mechanism of EDC is a complex process wherein the carbodiimides react with the carboxyl groups to form highly unstable species which further undergo reaction with the amine groups of the adjacent polypeptide chains to form a covalent amide linkage. The crosslinking efficiency is improved by employing NHS as a co-reactant. Studies have shown that fabrication of these high quality, optically clear and homogeneous hydrogels for clinical applications was difficult due to rapid gelation of collagen in the presence of EDC. Hence an alternative has been suggested to the above crosslinking system which assures prolonged gelation time and crosslinking at ambient temperatures. This new system employs *N*-cyclohexyl-*N′*-(2-morpholinoethyl) carbodiimide metho-*p*-toluenesulfonate (CMC) (Ahn, Kuffova et al. 2013). The presence of two bulky groups – cyclohexyl and 2-morpholinoethyl, across the diimide moiety, hinder the rapid crosslinking reaction due to steric hindrance. The CMC crosslinking enables production of hydrogels with properties comparable to those of the EDC crosslinked collagen-based materials. This crosslinked system, albeit tried to replicate the natural conditions of the corneal stroma, showed problems during in vivo grafting in the mouse cornea while performing pre-clinical trials. The hydrogel suffered from partial or complete extrusion from the graft bed owing to the softening of sutured areas. Moreover, clinically visible leak with the shallowing of the anterior chamber was distinctly evident. In spite of the above problems, the integrity of the anterior chamber was maintained by dense fibrovascular membrane developed behind the hydrogel. Nevertheless, this new system promises a bright future in regards to further research and can be exclusively scaled up for clinical applications owing to its unique properties.

2. RHC as a tissue engineered corneal substitute has been successfully fabricated in

the form of an optically clear, robust hydrogel (Liu, Merrett et al. 2008). A comparative study was carried out using recombinant human type I (larger diameter collagen fibrils) and type III (fine fibrils constituting reticular networks) collagen solutions crosslinked with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and N-hydroxysuccinimide (NHS). Results have shown that type III RHC hydrogels are superior in properties compared to type I. Type I and type III collagens are known to be distributed largely in various tissues and thus have been opted for the comparative study. They co-exist in the collagen fibrils. They showed higher or equal white light transmission compared to the human corneas (87%) (Beems and Van Best 1990). The crosslinking ratio of EDC/Coll-NH_2 did not influence the optical properties of these hydrogels significantly. Type III showed higher tensile strength and modulus than the type I for the same EDC/Coll-NH_2 ratios except at the ratio of 0.5. The diffusion coefficients for albumin and glucose for these hydrogels were the same as those for the human cornea. They showed excellent proliferation of corneal cells and nerves after a 12-month post-operative period. However, both the types supported in vitro epithelium and nerve over-growth in addition to the presence of essential requisites for surgical manipulation (Liu, Merrett et al. 2008). The former system and this one does not exhibit much difference except for the modification of collagen as stated before. RCH serve to eliminate the risk of pathogen transfer and xenogeneic immune-responses of animal collagen.

3. Collagen-phosphorylcholine interpenetrating network hydrogels are another class of modified collagen hydrogels. This system is developed from interpenetrating polymer networks of 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide and N-hydroxysuccinimide crosslinked porcine atelocollagen as one component and poly(ethylene glycol) diacrylate crosslinked 2-methacryloyloxyethyl phosphorylcholine (MPC) as the second component (Liu, Deng et al. 2009). MPC mimics the phospholipids which are found in the plasma membranes of the cells. MPC showed unique properties like anti-fouling, biocompatibility and high water holding capacity. Immobilization of MPC's onto the collagen imparted high mechanical properties and resistance to enzymatic degradation by collagenase (Goda, Watanabe et al. 2006). However, its anti-adhesive property did not favor cell attachment and its subsequent proliferation and differentiation. Thus to exploit its advantages without inculcating its limiting factor, an interpenetrating polymer network combining MPC and collagen was developed. These hydrogels assure to produce extracellular matrix (ECR) like environment thus sustaining corneal healing.

4.2 GELATIN BASED HYDROGELS

The collagen hydrogel systems face certain shortcomings like reduced transparency during wetting and hence demand improvements in its properties. This lead to the emergence and use of other alternatives like gelatin: a denatured form of collagen. Gelatin has been previously utilized on a massive scale in various biomedical and pharmaceutical applications owing to its appropriate biodegradability and biocompatibility in various physiological environment (Watanabe, Hayashi et al. 2011). Gelatin based hydrogels have been designed and exploited as cell carriers (corneal endothelium and stromal cells) and as an efficient ocular drug delivery system (Niu, Choi et al. 2014, Tsai, Hsu et al. 2014). These gelatin hydrogels exhibit reduced applications at higher temperatures (above 35^{o}C) due to breaking of the secondary bonding structure which sabotages the physical

network. This consequently leads to poor thermal and mechanical stability. Therefore, they have been chemically crosslinked to improve the gel stability and certain properties like elasticity and consistency (Dash, Foston et al. 2013, Hago and Li 2013). Gelatin has been modified by introducing functional groups in the acidic or basic type gelatin giving rise to anionized or cationized gelatin derivatives. These hydrogels are then doped with the epithelial growth factors (EGF) enabling controlled release (Hori, Sotozono et al. 2007). EGF is a soluble protein which facilitates proliferation of epithelial cells, fibroblasts etc. and is mainly secreted by the lachrymal gland. Thus to enhance the rate of corneal healing, exogenous EGF has been administered by various means. Unlike other polymeric gel-mediated delivery system where the drug retention time is restricted due to polymeric gel dilution and dispersion, these gelatin hydrogels prolong the release of GF. Out of 9 kinds of gelatin, cationized gelatin hydrogel (CGH) performed excellently as a carrier of EGF due to stable physiochemical interactions. Moreover, CGH has shown non-cytotoxicity and non-inflammatory reactions when applied to the cells cultured in vitro during pre-clinical trials. Although these systems haven't been fully commercialized and are still under stringent developmental schemes, they have opened new possibilities for the treatment of ocular surface diseases.

4.3 CHITOSAN BASED HYDROGELS

Chitosan belongs to a class of linear co-polymeric polysaccharides composed of $_D$-glucosamine and N-acetyl-$_D$-glucosamine by (1,4)-linkage. Chitosan has also been favored as a biomaterial for ocular drug delivery and ophthalmic formulations because of its biodegradability, biocompatibility, non-toxicity, antifungal and antimicrobial properties, penetration enhancing effect, uncanny drug loading capacity and cell implantation and excellent ocular tolerance (Cao, Zhang et al. 2007, Tsai, Hsu et al. 2014). Previously, poly (N-isopropylacrylamide) (PNIPAAm) hydrogels were used for the purpose of corneal healing; however it had been observed that they formed rigid and uncomfortable films on the cornea thereby hindering its potency for ocular applications. Incorporation of chitosan improved in-situ gel forming abilities in addition to the thermo-associative behavior. It combined the thermo-sensitive properties of PNIPAAm with the elasticity and robustness of chitosan giving birth to a novel hydrogel system which gave effective results for the treatment of glaucoma and other diseases (Cao, Zhang et al. 2007). Fabrication of poly (ethylene glycol) (PEG)-chitosan ultrathin hydrogel films (as suggested by Berkay et al.) supports proliferation and implantation of corneal endothelial cells (CEC). The dry, casted chitosan films were placed in solutions containing poly (ethylene glycol) diglycidyl ether (PEGDGE) and cystamine in the molar ratio of 4:1. The crosslinking of PEGDGE and cystamine follows an epoxy-amine chemistry. Following the epoxy-amine crosslinking, no further surface modification was carried out. These composite hydrogel films thus obtained are optically transparent and permeable to glucose and albumin, having mechanical properties that transcend that of the human cornea, albeit retaining the elastic properties. Above all, these films undergo in vitro degradation in the presence of lysozyme and L-cysteine within a time lapse of 8 weeks rendering them biodegradable which is the most essential necessity for any material to be employed in corneal regeneration (Ozcelik, Brown et al. 2013).

4.4 HYDROGEL ADHESIVES

Conventionally, corneal wounds are mended by means of nylon sutures. However, these sutures render the cornea vulnerable because of their tendency to cause increased trauma, corneal scarring due to persistent inflammation and vascularization, corneal epithelial erosion, irregular astigmatism and conjunctival edema (Shahinian and Brown 1977, Grinstaff 2007). Thus attempts have been made to replace the above procedure by the use of adhesives to fix the corneal wounds. These

adhesives must possess the following properties: (1) adequate viscosity (< 100 cP) to ensure controlled and rapid placement; (2) adherence to the moist corneal surface; (Patel, Marshall et al.) allow diffusivity of the nutrients; (4) maintain the structural integrity; (5) be biocompatible and antimicrobial; (6) possess the same refractive index as that of the human cornea and (7) be bio-absorbed or exuded from the wound as the tissue regeneration progresses (Grinstaff 2007). Cyanoacrylates have been extensively used in the past according to Webster et al. (Webster, Slansky et al. 1968) for closing of the corneal perforations. However, they have certain drawbacks like unwanted toxicity, inflexibility, inflammatory actions, lack of transparency and inability to be bio-absorbed leading to slower healing of the cells underneath (Bhatia 2006). A variety of such glues have been investigated over the years with the focus to obtain the most optimum adhesive system. Dendrimers, being one of them, are a class of unique macromolecules which are bestowed with extremely high flexibility and degree of branching with narrow molecular weight distribution. They show peculiar biological response, rheological properties and physical properties thereby assuring its applicability as an ocular tissue adhesive. Grinstaff et al. (Grinstaff 2007) presents a novel "bio-dendrimer" composed of generation four poly(glycerol-succinic acid) dendrimer with hydroxyl terminated groups ([G4]-PGLSA-OH), a generation three (([G3]-PGLSA-OH)$_2$-PEG) and a generation two lysine-cysteine Dendron. Here the generations refer to the layers of the dendrimers. Hydrogel adhesives can be obtained by successive crosslinking of these dendrimers. These hydrogels tend to conform to the requisites of an ideal adhesive. The crosslinking can be carried out in two ways: (1) modification of the terminal groups of the dendrimers into acrylates resulting in photo-crosslinking; (2) modification of the terminal groups to contain nucleophiles thereby causing crosslinking reactions with other polymers containing electrophiles as the terminal groups. These hydrogel adhesives are flexible, transparent, hydrophilic and can be easily used in conjunction with a reduced number of sutures. Dendrimeric architecture facilitates regulation over its structure and hence making it easy to obtain properties as per the requirement. Although corneal adhesives are far from clinical use, it is our responsibility to make the utmost use of their properties and develop a feasible market in the coming years.

5. SOME OTHER SCAFFOLDS

Apart from the above mentioned methods, some other methods have also been proposed and aggregated to the techniques of corneal regeneration. Studies have declared that Human Corneal Endothelial Cells (HCECs) show limited proliferative ability; hence they tend to exhaust quickly showing a very short life span with aging. Moreover, the rate of declination of these cells is further accelerated with the event of endothelial damage due to trauma, phacoemulsification and acute angle-closure glaucoma (Mimura, Yamagami et al. 2013). To counter the problems due to donor cornea, substitutes have been developed like chitosan based hydrogel discs (Ozcelik, Brown et al. 2013), gelatin based sterilized disks (Lai, Lu et al. 2006), amniotic membrane (Ishino, Sano et al. 2004), collagen sheets (Mimura, Yamagami et al. 2004), silk films (Lawrence, Marchant et al. 2009, Liu, Lawrence et al. 2012) and others on which cultured HCECs are transplanted to. The mentioned substrates act as an ideal scaffold with good biodegradability, non-cytotoxicity, transparency, permeability to water and nutrients, adequate mechanical properties, easily pliable and support HCEC growth (Niu, Choi et al. 2014).

5.1 GELATIN SCAFFOLD

In support to the above conjecture, Guoguang et. al. prepared thin gelatin gel (TGG) scaffolds functionalized with heparin to assist the transplantation of HCECs (Niu, Choi et al. 2014). The fabrication involves a two-step process: (1)

preparation of gelatin film by solution casting heparin sodium salt and gelatin solution and (2) crosslinking of the films by EDC/NHS combination. The incorporation of heparin in TGG loaded with basic fibroblast growth factor (bFGF) increased the proliferation of the HCECs. These bFGFs are responsible for the improved survival of the HCECs reducing the cellular loss (Rieck, Oliver et al. 1995). These modified TGG scaffolds showed high transparency and flexibility allowing them to be implanted in the anterior chamber of the eye through a modicum incision. They exhibited benign adherence to their corneal stroma without any signs of inflammation and successfully mimicked the bio-environment.

5.2 COLLAGEN-GELATIN SCAFFOLD

Yang Liu et. al. developed a novel biomimetic film of crosslinked collagen-gelatin-hyaluronic acid, in the ratio 6:3:1, with the aim of incorporating the unique properties of each component giving birth to a more better scaffold for corneal tissue engineering (Liu, Ren et al. 2013). Collagen is the most extensively used biomaterial and thus forms the main load bearing component in the connective tissues (Li, Griffith et al. 2005, Liu, Merrett et al. 2008, Liu, Deng et al. 2009, Builles, Janin-Manificat et al. 2010, Ahn, Kuffova et al. 2013). Gelatin has been preferred for its biocompatibility and antigenicity (Hori, Sotozono et al. 2007, Watanabe, Hayashi et al. 2011) and hyaluronic acid, being one of the cardinal components of the ECM, enables the adhesion and proliferation of CECs (Frantz, Stewart et al. 2010). The crosslinking was achieved via the usual EDC/NHS (1:1) combination. These films gave excellent outcome in terms of optical transparency, biocompatibility, mechanical properties and diffusion of nutrients, almost comparable to the human cornea.

5.3 PEPTIDE NANOFIBRE SCAFFOLD

Peptides are useful for creating self-assembling nanostructure owing to their inherent biocompatibility and biodegradability. β sheet forming peptides have the ability to assemble into one dimensional nanostructure through intermolecular hydrogen bonding and interactions among these one dimensional structures can lead to the formation of three dimensional networks. Peptides can be tuned by incorporation of specific amino acid sequences which can help to mimic the structure and function of native extracellular matrix (Cui, Webber et al. 2010). Peptide amphiphiles [PA] are a class of self-assembling biomaterials. PAs have a short hydrophobic block, generally an alkyl chain incorporated on the end of a short peptide sequence. They can be developed so as to self-assemble under specific conditions of pH, temperature or ionic strength. Epitopes that promote cell adhesion are frequently incorporated in PAs, mimicking the desired ECM property of adhesion (Cui, Webber et al. 2010). Ricardo M. Gouveia et al. created two synthetic PAs containing bioactive epitopes, the $C_{16}G_3RGD$ (RGD) and $C_{16}G_3RGDS$ (RGDS), as biocompatible film coatings to enhance adhesion, proliferation, and alignment of human corneal stromal fibroblasts while inducing the formation of 3D lamellar-like stromal tissue in the absence of serum (Gouveia, Castelletto et al. 2013). G.Uzunalli et al. compared the PAs containing YIGSR and RGD epitopes. YIGSR sequence is derived from laminin β chain and RGD from fibronectin. The PA solutions can be injected in the corneal pocket and as they are positively charged, they self-assemble in the presence of negatively charged molecules like chondroitin sulfate and keratin sulfate present in the cornea. Human corneal keratocyte cells cultured on laminin mimetic YIGSR nanofibers showed enhanced proliferation. Also there were no changes observed in the corneal morphology. Injection of YIGSR-PA gel into the corneal stromal pocket showed keratocyte migration and collagen-I expression, enhancing stroma regeneration three weeks after the surgery. RGD-PA nanofibers did not show a similar effect. Injectable peptide nanofiber scaffolds have an edge over other regenerative techniques as they allow for

flexibility in application in corneal tissue (Uzunalli, Soran et al. 2014).

6. CONCLUSION

Parallel to the advances in keratoplasty techniques there has been extensive research on synthetic and natural biomaterials. The progress in biomedical polymer science has hugely contributed to rapid development in corneal regeneration methods. The challenge which still remains is to create an alternative which has the combined desired properties such as mechanical strength, optical transparency, biocompatibility and which not only mimics the cornea at a micro environment level but also at a nano-environment level. Also further studies and follow ups are required for precise knowledge of cellular response to these matrices. Even with all the challenges, the future of corneal regeneration is indeed promising. By combining our understanding of corneal tissue, biomaterials and cell regeneration, better and improved therapies can be expected in the near future.

7. REFERENCES

1. Ahearne, M., K. K. Liu, A. J. El Haj, K. Y. Then, S. Rauz and Y. Yang (2010). "Online monitoring of the mechanical behavior of collagen hydrogels: influence of corneal fibroblasts on elastic modulus." Tissue Eng Part C Methods **16**(2): 319-327.
2. Ahn, J. I., L. Kuffova, K. Merrett, D. Mitra, J. V. Forrester, F. Li and M. Griffith (2013). "Crosslinked collagen hydrogels as corneal implants: effects of sterically bulky vs. non-bulky carbodiimides as crosslinkers." Acta Biomater **9**(8): 7796-7805.
3. Beems, E. M. and J. A. Van Best (1990). "Light transmission of the cornea in whole human eyes." Exp Eye Res **50**(4): 393-395.
4. Bhatia, S. S. (2006). "Ocular surface sealants and adhesives." Ocul Surf **4**(3): 146-154.
5. Builles, N., H. Janin-Manificat, M. Malbouyres, V. Justin, M. R. Rovere, G. Pellegrini, J. Torbet, D. J. Hulmes, C. Burillon, O. Damour and F. Ruggiero (2010). "Use of magnetically oriented orthogonal collagen scaffolds for hemi-corneal reconstruction and regeneration." Biomaterials **31**(32): 8313-8322.
6. Cao, Y., C. Zhang, W. Shen, Z. Cheng, L. L. Yu and Q. Ping (2007). "Poly(N-isopropylacrylamide)-chitosan as thermosensitive in situ gel-forming system for ocular drug delivery." J Control Release **120**(3): 186-194.
7. Chirila, T. V. (2001). "An overview of the development of artificial corneas with porous skirts and the use of PHEMA for such an application." Biomaterials **22**(24): 3311-3317.
8. Cui, H., M. J. Webber and S. I. Stupp (2010). "Self-assembly of peptide amphiphiles: from molecules to nanostructures to biomaterials." Biopolymers **94**(1): 1-18.
9. Dash, R., M. Foston and A. J. Ragauskas (2013). "Improving the mechanical and thermal properties of gelatin hydrogels cross-linked by cellulose nanowhiskers." Carbohydr Polym **91**(2): 638-645.
10. DelMonte, D. W. and T. Kim (2011). "Anatomy and physiology of the cornea." J Cataract Refract Surg **37**(3): 588-598.
11. Deshpande, P., C. Ramachandran, F. Sefat, I. Mariappan, C. Johnson, R. McKean, M. Hannah, V. S. Sangwan, F. Claeyssens, A. J. Ryan and S. MacNeil (2013). "Simplifying corneal surface regeneration using a biodegradable synthetic membrane and limbal tissue explants." Biomaterials **34**(21): 5088-5106.
12. Dohlman, C. H. and M. G. Doane (1994). "Some Factors Influencing Outcome After Keratoprosthesis Surgery." Cornea **13**(3): 214-218.
13. Elisseeff, J., M. G. Madrid, Q. Lu, J. J. Chae and Q. Guo (2013). "Future Perspectives for Regenerative Medicine in Ophthalmology." Middle East African Journal of Ophthalmology **20**(1): 38-45.
14. Frantz, C., K. M. Stewart and V. M. Weaver (2010). "The extracellular matrix at a glance." J Cell Sci **123**(Pt 24): 4195-4200.
15. Goda, T., J. Watanabe, M. Takai and K. Ishihara (2006). "Water structure and improved mechanical properties of phospholipid polymer hydrogel with phosphorylcholine centered intermolecular cross-linker." Polymer **47**(4): 1390-1396.
16. Gouveia, R. M., V. Castelletto, S. G. Alcock, I. W. Hamley and C. J. Connon (2013). "Bioactive films produced from self-assembling peptide amphiphiles as versatile substrates for tuning cell adhesion and tissue architecture in serum-free conditions." Journal of Materials Chemistry B **1**(44): 6157-6169.
17. Grinstaff, M. W. (2007). "Designing hydrogel adhesives for corneal wound repair." Biomaterials **28**(35): 5205-5214.
18. Hago, E.-E. and X. Li (2013). "Interpenetrating Polymer Network Hydrogels Based on Gelatin and PVA by Biocompatible Approaches: Synthesis and Characterization." Advances in Materials Science and Engineering **2013**: 8.
19. Hayashida, Y., K. Nishida, M. Yamato, K. Watanabe, N. Maeda, H. Watanabe, A. Kikuchi, T. Okano and Y. Tano (2005). "Ocular surface reconstruction using autologous rabbit oral mucosal epithelial sheets fabricated ex vivo on a temperature-responsive culture surface." Invest Ophthalmol Vis Sci **46**(5): 1632-1639.
20. Hicks, C. R., J. H. Fitton, T. V. Chirila, G. J. Crawford and I. J. Constable (1997). "Keratoprostheses: advancing toward a true artificial cornea." Surv Ophthalmol **42**(2): 175-189.
21. Hori, K., C. Sotozono, J. Hamuro, K. Yamasaki, Y. Kimura, M. Ozeki, Y. Tabata and S. Kinoshita (2007). "Controlled-release of epidermal growth factor from cationized gelatin hydrogel enhances corneal epithelial wound healing." J Control Release **118**(2): 169-176.

22. Ishino, Y., Y. Sano, T. Nakamura, C. J. Connon, H. Rigby, N. J. Fullwood and S. Kinoshita (2004). "Amniotic membrane as a carrier for cultivated human corneal endothelial cell transplantation." Invest Ophthalmol Vis Sci **45**(3): 800-806.
23. Lai, J.-Y. and G.-H. Hsiue (2007). "Functional biomedical polymers for corneal regenerative medicine." Reactive and Functional Polymers **67**(11): 1284-1291.
24. Lai, J. Y., P. L. Lu, K. H. Chen, Y. Tabata and G. H. Hsiue (2006). "Effect of charge and molecular weight on the functionality of gelatin carriers for corneal endothelial cell therapy." Biomacromolecules **7**(6): 1836-1844.
25. Lawrence, B. D., J. K. Marchant, M. Pindrus, F. Omenetto and D. L. Kaplan (2009). "Silk film biomaterials for cornea tissue engineering." Biomaterials **30**(7): 1299-1308.
26. Lee, C. H., A. Singla and Y. Lee (2001). "Biomedical applications of collagen." Int J Pharm **221**(1-2): 1-22.
27. Li, F., M. Griffith, Z. Li, S. Tanodekaew, H. Sheardown, M. Hakim and D. J. Carlsson (2005). "Recruitment of multiple cell lines by collagen-synthetic copolymer matrices in corneal regeneration." Biomaterials **26**(16): 3093-3104.
28. Liu, J., B. D. Lawrence, A. Liu, I. R. Schwab, L. A. Oliveira and M. I. Rosenblatt (2012). "Silk fibroin as a biomaterial substrate for corneal epithelial cell sheet generation." Invest Ophthalmol Vis Sci **53**(7): 4130-4138.
29. Liu, W., C. Deng, C. R. McLaughlin, P. Fagerholm, N. S. Lagali, B. Heyne, J. C. Scaiano, M. A. Watsky, Y. Kato, R. Munger, N. Shinozaki, F. Li and M. Griffith (2009). "Collagen-phosphorylcholine interpenetrating network hydrogels as corneal substitutes." Biomaterials **30**(8): 1551-1559.
30. Liu, W., K. Merrett, M. Griffith, P. Fagerholm, S. Dravida, B. Heyne, J. C. Scaiano, M. A. Watsky, N. Shinozaki, N. Lagali, R. Munger and F. Li (2008). "Recombinant human collagen for tissue engineered corneal substitutes." Biomaterials **29**(9): 1147-1158.
31. Liu, Y., L. Ren and Y. Wang (2013). "Crosslinked collagen-gelatin-hyaluronic acid biomimetic film for cornea tissue engineering applications." Mater Sci Eng C Mater Biol Appl **33**(1): 196-201.
32. Mimura, T., S. Yamagami and S. Amano (2013). "Corneal endothelial regeneration and tissue engineering." Prog Retin Eye Res **35**: 1-17.
33. Mimura, T., S. Yamagami, S. Yokoo, T. Usui, K. Tanaka, S. Hattori, S. Irie, K. Miyata, M. Araie and S. Amano (2004). "Cultured human corneal endothelial cell transplantation with a collagen sheet in a rabbit model." Invest Ophthalmol Vis Sci **45**(9): 2992-2997.
34. Nishida, K., M. Yamato, Y. Hayashida, K. Watanabe, K. Yamamoto, E. Adachi, S. Nagai, A. Kikuchi, N. Maeda, H. Watanabe, T. Okano and Y. Tano (2004). "Corneal reconstruction with tissue-engineered cell sheets composed of autologous oral mucosal epithelium." N Engl J Med **351**(12): 1187-1196.
35. Niu, G., J. S. Choi, Z. Wang, A. Skardal, M. Giegengack and S. Soker (2014). "Heparin-modified gelatin scaffolds for human corneal endothelial cell transplantation." Biomaterials **35**(13): 4005-4014.
36. Ozcelik, B., K. D. Brown, A. Blencowe, M. Daniell, G. W. Stevens and G. G. Qiao (2013). "Ultrathin chitosan-poly(ethylene glycol) hydrogel films for corneal tissue engineering." Acta Biomater **9**(5): 6594-6605.
37. Parenteau-Bareil, R., R. Gauvin and F. Berthod (2010). "Collagen-based biomaterials for tissue engineering applications." Materials **3**(3): 1863-1887.
38. Patel, S., J. Marshall and F. W. Fitzke, 3rd (1995). "Refractive index of the human corneal epithelium and stroma." J Refract Surg **11**(2): 100-105.
39. Rieck, P., L. Oliver, K. Engelmann, G. Fuhrmann, C. Hartmann and Y. Courtois (1995). "The role of exogenous/endogenous basic fibroblast growth factor (FGF2) and transforming growth factor beta (TGF beta-1) on human corneal endothelial cells proliferation in vitro." Exp Cell Res **220**(1): 36-46.
40. Ruberti, J. W., A. S. Roy and C. J. Roberts (2011). "Corneal biomechanics and biomaterials." Annu Rev Biomed Eng **13**: 269-295.
41. Said, D. G., M. Nubile, T. Alomar, A. Hopkinson, T. Gray, J. Lowe and H. S. Dua (2009). "Histologic features of transplanted amniotic membrane: implications for corneal wound healing." Ophthalmology **116**(7): 1287-1295.
42. Shahinian, L., Jr. and S. I. Brown (1977). "Postoperative complications with protruding monofilament nylon sutures." Am J Ophthalmol **83**(4): 546-548.
43. Tsai, I. L., C. C. Hsu, K. H. Hung, C. W. Chang and Y. H. Cheng (2014). "Applications of biomaterials in corneal wound healing." J Chin Med Assoc.
44. Tsai, R. J., L. M. Li and J. K. Chen (2000). "Reconstruction of damaged corneas by transplantation of autologous limbal epithelial cells." N Engl J Med **343**(2): 86-93.
45. Uzunalli, G., Z. Soran, T. S. Erkal, Y. S. Dagdas, E. Dinc, A. M. Hondur, K. Bilgihan, B. Aydin, M. O. Guler and A. B. Tekinay (2014). "Bioactive self-assembled peptide nanofibers for corneal stroma regeneration." Acta Biomater **10**(3): 1156-1166s.
46. van Wachem, P. B., J. A. Plantinga, M. J. Wissink, R. Beernink, A. A. Poot, G. H. Engbers, T. Beugeling, W. G. van Aken, J. Feijen and M. J. van Luyn (2001). "In vivo biocompatibility of carbodiimide-crosslinked collagen matrices: Effects of crosslink density, heparin immobilization, and bFGF loading." J Biomed Mater Res **55**(3): 368-378.
47. Watanabe, R., R. Hayashi, Y. Kimura, Y. Tanaka, T. Kageyama, S. Hara, Y. Tabata and K. Nishida (2011). "A novel gelatin hydrogel carrier sheet for corneal endothelial transplantation." Tissue Eng Part A **17**(17-18): 2213-2219.
48. Webster, R. G., Jr., H. H. Slansky, M. F. Refojo, S. A. Boruchoff and C. H. Dohlman (1968). "The use of adhesive for the closure of corneal perforations. Report of two cases." Arch Ophthalmol **80**(6): 705-709.
49. Whitcher, J. P., M. Srinivasan and M. P. Upadhyay (2001). "Corneal blindness: a global perspective." Bull World Health Organ **79**(3): 214-221.
50. Wright, B., S. Mi and C. J. Connon (2013). "Towards the use of hydrogels in the treatment of limbal stem cell deficiency." Drug Discov Today **18**(1-2): 79-86.
51. Yamato, M. and T. Okano (2004). "Cell sheet engineering." Materials today **7**(5): 42-47.
52. Yang, J., M. Yamato, C. Kohno, A. Nishimoto, H. Sekine, F. Fukai and T. Okano (2005). "Cell sheet engineering: recreating tissues without biodegradable scaffolds." Biomaterials **26**(33): 6415-6422.