Eye (2009) 23, 1985–1989

www.nature.com/eye

CAMBRIDGE OPHTHALMOLOGY SYMPOSIUM

Artificial corneas: a regenerative medicine approach

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Abstract

Corneal substitutes are being developed to address the shortage of human donor tissues as well as the current disadvantages in some clinical indications, which include immune rejection. In the past few years, there have been significant developments in bioengineered corneas that are designed to replace part or the full thickness of damaged or diseased corneas that range from keratoprostheses that solely address the replacement of the cornea's function, through tissue-engineered hydrogels that permit regeneration of host tissues. We describe examples of corneal substitutes that encourage regeneration of the host tissue. We also contend that it is unlikely that there will be a single "one-size-fits-all" corneal substitute for all indications. Instead, there will most likely be a small range of corneal substitutes ranging from prostheses to tissue-engineered matrix substitutes that are tailored to different clusters of clinical indications. The tissue-engineered matrices can either be produced as sterile acellular matrices, or complete with functional cells, ready for implantation.
Eye (2009) **23,** 1985–1989; doi:10.1038/eye.2008.409; published online 16 January 2009

Keywords: corneal substitutes; transplantation; regeneration

Introduction

The human cornea can be viewed as an optically clear hydrogel (hydrated gel) comprising mostly collagen, proteoglycans, and cells. This hydrogel or stromal compartment is sandwiched between an outer, stratified, non-keratinized epithelium, and an inner, single-layered endothelium.[1] The optical clarity of the cornea is now attributed to a combination of refractive index matching and the presence of structural components well below the wavelength of visible light.[2,3] Another unique property of the cornea is that it is avascular as well as mostly immune privileged.[4]

Loss of optical clarity occurs when the cornea is damaged by injury or disease. When irreversible, blindness occurs. To date, the only widely accepted treatment is transplantation with human donor corneas. However, in many countries, the demand for good quality tissue exceeds the supply. In addition, there are conditions that are not amenable for donor transplantation. Hence, artificial corneal substitutes are needed to address the shortage of human donor tissues as well as the current disadvantages in some clinical indications, which include immune rejection. They range from fully synthetic prostheses to tissue-engineered hydrogels that allow varying degrees of regeneration of the host tissues.

There have been several very recent, comprehensive reviews on artificial corneas including reviews from the present authors.[5–8] This study, therefore, is not meant as a review of the subject. Rather, our aim is to focus on the regenerative medicine approach to corneal transplantation, and in particular our own research progress, and those of others in the field in regenerating damaged corneas.

Keratoprostheses (KPros): completely synthetic corneal replacements

Although not a focus of this study, it would be pertinent to very briefly discuss corneal prostheses or KPros as these are the traditional and best-known 'artificial corneas'. They are mainly designed to restore a functional level of vision rather than to regenerate the cornea. The majority is made from plastic polymers and is designed to have a transparent central optic surrounded by a skirt to provide stable anchorage through the integration into the host tissue. Most KPro skirts are designed to be porous and to promote cellular integration of the host tissue through fibroblast in-growth. Newer designs that incorporate extracellular

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Received: 2 December 2008
Accepted in revised form: 2 December 2008
Published online: 16 January 2009

This work was presented at the Cambridge Ophthalmological Symposium

matrix (ECM) surface deposition to provide firm anchorage have been reported.[9–11] Various materials have been employed in skirt design, maintaining proper strength to allow for suture placement. Some newer KPro designs now have modified anterior surfaces to promote epithelialization, as well methods to inhibit downgrowth of epithelial cells into the stroma/implant interface. As well, the posterior surface can be modified to inhibit cellular attachment and proliferation to prevent retroprosthetic membrane formation, which leads to corneal opacification. Other innovations include considerations to allow sufficient oxygen permeation and nutrient permeability to maintain the cellular components.[12] Most importantly, however, the synthetic materials used in the device should be neither immunogenic nor mutagenic to allow graft acceptance.

Self-assembled corneal substitutes

Several groups have been working on self-assembled corneal substitutes in which corneal cells are induced by ascorbic acid or derivatives of ascorbic acid to secrete ECM macromolecules to produce corneal 'stromas'. Gaudreault *et al*,[13] for example, used ascorbic acid to promote the production of collagen and other ECM molecules by dermal fibroblast cells. The resulting ECM sheets are then stacked together to form a stroma, and allowed to further integrate in culture, after which an epithelium is seeded on top of the stack. Such constructs have been reported to show excellent corneal morphology. The cells also expressed the appropriate tissue-specific markers. The primary drawback of this method is the time needed to produce enough transplantable material rapidly for transplantation. More recently, Carrier *et al*[14] reported a new self-assembled model consisting of a mixed human corneal and dermal fibroblasts stroma. The authors contend that the combination of the corneal and dermal fibroblasts was more conducive to the formation of a well-differentiated epithelium that showed higher re-epithelialization rates than just corneal fibroblasts alone. This model was able to reproduce the microanatomy of the native human cornea as well as a mechanistically accurate wound-healing process. The authors suggest that this model would be useful as a tool for studying wound healing and screening of bioactive factors that could modulate wound healing.[14]

Guo *et al*,[15] on the other hand, induced primary human corneal fibroblasts to secrete ECM for the development of corneal substitutes. The average culture took 4 weeks to produce a multilayered, highly cellular construct of about 36-μm thickness. The cells in these constructs were arranged in parallel layers, similar to that of the mammalian corneal stroma. Secreted collagen fibrils ranged on average, between 27 and 51 nm, with a mean of 38.1 ± 7.4 nm, compared to the 31 ± 0.8 nm reported in adult human corneas.[16] This approach is gaining popularity, especially after the discovery of stem or progenitor cell populations within the stroma.[17,18] Such an approach is very promising as it potentially allows the *in vitro* reconstruction of corneal tissue from autologous sources of stem cells.

Acellular tissue-engineered scaffolds to promote endogenous regeneration

It is known that the ECM macromolecules have a role in guiding cellular growth and differentiation during embryonic development. They can, therefore, be tapped for use in promoting regeneration. As type I collagen is the predominant ECM macromolecule found in the human cornea (70% dry weight), it has been investigated by a number of groups for its use as a scaffold for artificial cornea construction.

Type I collagen scaffolds have been used to cultivate human corneal stromal fibroblasts *in vitro*. In these cultures, the cell-scaffold interactions resulted in changes in the mechanical and permeability properties of the gels.[19,20] These results showed that stromal cells were able to modulate their own environments, by remodelling the matrix support and changing the properties more to that of a natural stroma. In fact, the resultant tissue had a lamellar-like microstructure following 21 days of incubation compared to its initial spongy structure.

The authors have developed various collagen-based corneal substitutes that have been implanted successfully into a range of animal models including mice, rabbits, and pigs as either deep lamellar grafts, or full-thickness implants. These hydrated gels or hydrogels can be fabricated to the appropriate dimensions and curvatures, which allow for transmission of 90% or higher of white light. Collagen sources have included both porcine and bovine-extracted collagens, as well as the more current use of recombinant human collagen. To make the gels mechanically strong enough to permit suturing, and resist biodegradation, cross-linking, copolymerization, and development of interpenetrating networks have been used. The simplest hydrogel we developed was a collagen-based cornea stroma mimic fabricated by cross-linking collagen with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) that could be successfully implanted into minipigs with stable host–graft integration. Most recently, fibrillar recombinant human collagens, types I (RHCI) and III (RHCIII), were examined as corneal stromal matrix substitutes in pigs.[21,22] At 12 months of postimplantation, both RHC I

and III implants were integrated and optically clear. The acellular implants had been overgrown with a stratified epithelium that was stably anchored. There was also in-growth of stromal cells and corneal sensory nerves.[21,22] The advantage of RHC is that it is produced synthetically in yeast and therefore avoids the risk of disease transmission from animal-extracted collagen and possible immune response. Although properties of both RHCI and RHCIII were fairly similar, the optical properties of RHCIII were better and showed a trend towards higher mechanical strength. Hydrogels with starting RHC concentrations of 13.7% yielded constructs with mechanical properties of up to 1.7 MPa tensile strength with type III recombinant human collagen, with 14% elongation at break and an elastic modulus of about 20 MPa.[21] The human cornea by comparison is 3.8 MPa in tensile strength,[21] with an elastic modulus of 3–13 MPa.[21] We also noted that the remodelling of the pig corneas had occurred and the cornea stromas at 12 months postoperative show a lamellar arrangement at both light and TEM levels.

Currently, phase I human clinical trials have begun in Sweden where RHCIII corneal substitutes were implanted as deep anterior lamellar grafts into 10 patients, aged 19–76 years, with either keratoconus or central scarring from bacterial keratitis.[23] Figure 1 shows slit-lamp images from a patient after the implantation, after suture removal (at 4–5 weeks postoperative), and at 6 and 9 months postoperative. The epithelium had regenerated (Figure 2a) in all 10 patients, and stromal cells had grown into the implant (Figure 2b) to anchor the grafts. Fine subepithelial nerves were also observed (Figure 2a) beginning at 3 months postoperative in a couple of younger patients. To date, we have shown that the implants do not cause adverse reactions, and therefore are suitable as temporary grafts or patches. However, longer-term monitoring and testing in a larger patient population is needed to determine whether or not they will be useful as substitutes for donor tissue. In addition, further modifications are likely needed to be useful to a wider range of clinical indications.

We have also shown that synthetic materials can be combined with our collagen-based corneal alternatives to enhance the interaction with the host cornea or to strengthen the construct. By incorporating an artificial polymer, poly(N-isopropylacrylamide-coacrylic acid-coacryloxysuccinimide) into our collagen corneal alternatives, we were able to modify the gels to include the laminin-derived pentapeptide, YIGSR, into the matrix.[24] Upon deep lamellar keratoplasty implantation into minipigs, we showed that incorporation of YIGSR into the corneal implant greatly enhanced the rate of nerve regeneration, for example, restoration of corneal touch sensitivity within a 6-week period,

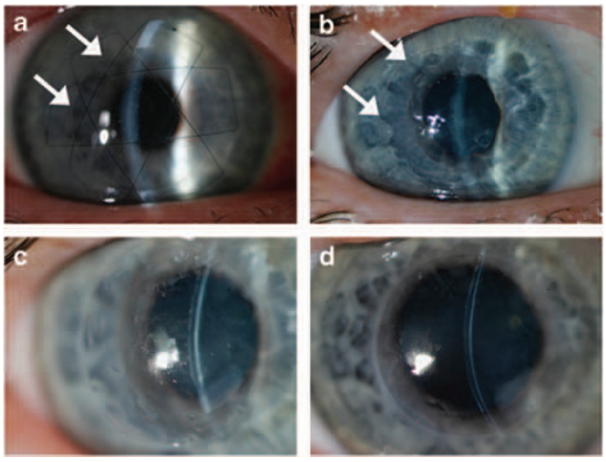

Figure 1 Slit-lamp images from a 37-year-old male patient (a) after implantation; (b) after suture removal at 1-month post-operative; (c) at 6-month postoperative; and (d) at 9-month postoperative. Arrow in (a) and (b) indicate the graft–host interface.

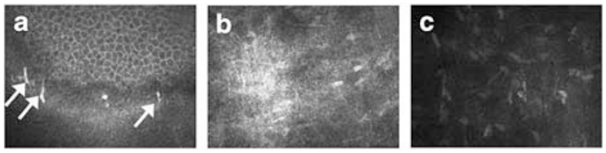

Figure 2 *In vivo* confocal images from a 19-year-old male patient at 6-month postoperative. (a) Epithelium showing regenerating nerves (arrows). (b) The bright background represents a site of high activity, where activated stromal cells are infiltrating the implant. (c) Host stromal cells that have populated the implant.

compared with allografts, which remained non-responsive to touch.[24]

We also show that corneal substitutes can be fabricated to incorporate micro- or nanoparticles that would release a drug.[25] Additional interpenetrating networks of biointeractive materials that retain stability in potentially adverse disease environments, for example, where high levels of enzymes are produced are also being developed.[26]

Tissue-engineered scaffolds for stem and progenitor cell delivery

In many cases, only one corneal layer, usually the epithelial layer, which is exposed to the environment, is damaged. In cases such as chemical burns or dry eye syndrome, the stem and progenitor cells that are normally responsible for affecting the repair may also be decimated. Hence, various groups have been developing stem cell-based methods for repopulating the cornea.

Reconstruction of the epithelium can occur through corneal stem cells from the surrounding limbus either

from the undamaged contralateral eye (autograft) or from allogeneic sources. The explants are most frequently seeded on prepared human amniotic membranes[27] or fibrin substrates,[28] and outgrowing cells are allowed to form sheets that are transplanted onto the damaged eye. More recently, the authors and other collaborators have seeded corneal limbal cells onto fully synthetic, cross-linked human recombinant collagen type I and type III substrates and have shown that fully stratified corneal epithelia can be reconstituted on these substrates.[29]

In some patients, where both corneal surfaces in both eyes have been depleted of stem cells, for example, 12 patients with Stevens–Johnson syndrome, chemical and thermal injury, pseudoocular cicatricial pemphigoid, and idiopathic ocular surface disorder, successful autologous reconstruction of the corneal surface by transdifferentiation of oral mucosal epithelium has been performed by Kinoshita and colleagues,[30] Kinoshita and colleagues,[30] further suggest that the use of transdifferentiated autologous epithelial precursor cells may be safer for ocular resurfacing than with allogeneic grafts in particular younger patients with the most severe ocular surface disorders. However, it was noted that all transplanted eyes had some peripheral corneal neovascularization.

Similar stem cell-based strategies are being tested for corneal endothelial replacements. However, these are not as well advanced as the epithelial replacement strategies.[31]

Reconstructions of complete human corneal constructs with all three cell layers has were reported by our group using immortalized cell lines and a glutaraldehyde cross-linked collagen–chondroitin sulphate hydrogel scaffold for toxicology testing.[32] Recently, Vrana *et al*[33] reported an adaptation of this scaffold, in the form of a foam that used a zero-length carbodiimide crosslinker, which would not be incorporated into the scaffold, for reconstruction of a corneal model designed for use in transplantation. In addition, they replaced the epithelial and stromal cell lines with primary and progenitor cells, in anticipation of further development towards a clinically applicable model.

Conclusion

We have shown several examples of how a regenerative medicine approach to developing corneal substitutes is gaining popularity. Several of these strategies are now in the clinics or in clinical trials. In general, corneal alternatives must be able to reproduce the desired function of the initial tissue, for example, optical properties. The use of biomaterials, either naturally secreted by stimulation (eg, with ascorbic acid), fabricated by tissue engineering, or simply using naturally occurring membranes, for example, amniotic membranes, have been shown to enhance cell adhesion, proliferation, and differentiation. In some conditions, endogenous repair is possible, for example, by use of biointeractive acellular scaffolds that promote endogenous precursor cells to affect the repair. In other cases, there is need to induce the repair process by the delivery of stem cells to initiate the regenerative process.

Acknowledgements

Funding for research conducted in the authors' laboratoriess is from NSERC and CIHR, Canada, the Canadian Stem Cell Network, the Swedish Research Council, and the County of Östergötland, Sweden. We thank Cooper Vision for fabrication of the corneal implants for the phase I clinical study, and various colleagues in Canada, USA, Japan, China, Taiwan, India, and the EU for their previous and continuing, invaluable contributions to this project.

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