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# Artificial Corneas, and Reinforced Composite Implants for High Risk Donor Cornea Transplantation

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### Abstract

Here, we review examples of artificial corneas that have been developed as alternatives to donor cornea transplantation. These consist of artificial corneas developed as prostheses and regenerative scaffolds. Examples of reinforced and composite implants developed within our group are profiled.

**Keywords:** Cornea, Regeneration, Biomaterials, Implants, HSV-1.

## 8.1 Introduction

### 8.1.1 Cornea Transplantation

In Sweden, as in most countries of the world, there is a serious shortage of donor organs for transplantation, and when organs are available, there is still a problem of immune rejection. The myth is that the cornea is an immune privileged site and hence transplantation is problem-free. However, in reality, there are many pathological conditions where the immune privilege is gone (e.g., chemical burns, severe/persistent viral and bacterial infections, severe dry eye, neuropathic issues, autoimmune conditions, etc.). In addition, while

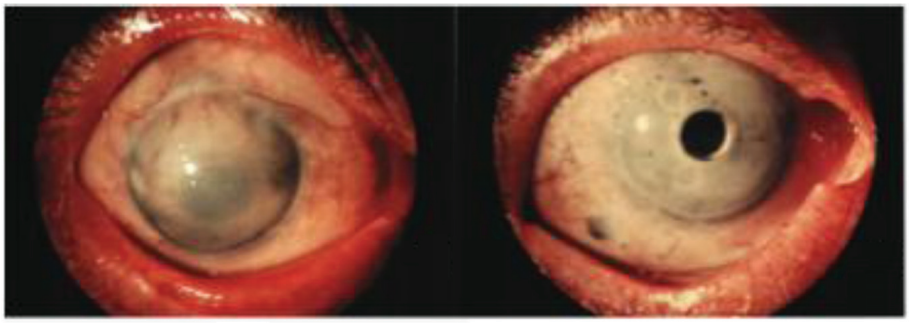
the two year success rates for cornea allografting are high – 85% in a developed EU nation like Sweden [1], the long-term success at 10–15 years falls to 55% [2], which is even lower than that for kidney transplantation [3]. In a developing country, however, even the short term rates start out low, at 69% in South India [4]. This is most likely due to the higher incidence of more severe pathologies such as chemical burns or inflammation. In the cornea, as in other solid organs, graft rejection is directed against the foreign cells, and this is managed by steroid use for 6 to 12 months, or even 2–3 years.

Worldwide, an estimated 10 million individuals are in need of corneal transplantation, but there is a severe shortage of good quality donor corneas. Disease transmission is pre-empted by very rigorous and expensive screening but still transmission of diseases such as HSV, rabies and Creutzfeldt-Jakob disease have been traced back to the donor corneas.

## 8.2 Artificial Corneas as Prostheses and Regeneration Templates

### 8.2.1 Artificial Corneas as Prostheses

In the cornea transplantation, the use of allogeneic donor corneas has remained the 'gold standard' or state-of-the-art for over a century despite numerous efforts to develop synthetic prostheses (called keratoprostheses or KPros). There have been many attempts to produce the optimal biomaterials to fabricate KPros. Most have resulted in a high proportion of rejection and need sustained immunosuppression but several are very successful and are the only options for corneas that are very badly damaged or diseased. The most successful prostheses and amongst the best known prostheses, the Boston KPro and Osteo-odonto-keratoprosthesis (OOKP) both have a biological interface. The former uses a human corneal rim as an interface while the latter uses a layer of oral mucosal cells that is wrapped around the tooth or bone implant that holds the acrylic optic. Another successful KPro that has been used since 1978 is the Filatov Institute K-Pro, which is usually implanted in leukomas that have been previously strengthened with oral mucosa or human donor corneas (Figure 8.1a–b). This device consists of an acrylic optic with tantalum support (Figure 8.1c). A cartilage graft taken from the ear or human donor corneal allograft consisting of posterior stromal layers and Descemet's membrane is implanted together with the Filatov Institute K-Pro to enhance stability of the device in corneal layers [5].



(a) (b)

(c)

**Figure 8.1** Example of Filatov Institute KPro used in a patient with avascular leukoma. a) Preoperatively, the cornea was in the terminal stage of bullous keratopathy, aphakia, having been previously operated on for secondary glaucoma; b) After keratoprosthesis implantation, there was simultaneous intracorneal strengthening of the leukoma with lamellar corneal allograft. Visual acuity was 12/20. Follow-up period was 16 years; c) The Iakymenko-Golubenko KPro, also referred to as the "universal dismountable" KPro; developed and being used at Filatov Inst. since 1978.

Although KPros are well-retained and the success rate is improving, several KPro models still suffer from the drawbacks of complex implantation procedures and serious complications, including retroprosthetic membrane formation, calcification, infection, glaucoma, and retinal detachment. Their use is therefore limited to cases in which allogeneic tissue has failed repeatedly or is contraindicated.

### 8.2.2 Artificial Corneas as Regeneration Templates

The first artificial cornea that was designed as a 'regeneration template' to promote regeneration of cornea tissue and nerves was reported in Fagerholm et al. (2010) [6]. We successfully completed the first-in-man Phase I clinical study in which cell-free biomimetic corneal implants made from 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC)/N- hydroxysuccinimide (NHS) crosslinked recombinant human collagen type III (RHCIII) were used to replace the pathologic anterior cornea of ten patients with significant vision loss (from advanced keratoconus and scarring). Once in the patient, the cell-free implants stimulated the patient's own endogenous corneal cells to migrate into the scaffolds, regenerate, producing corneal tissue and nerve regeneration and vision improvement. On its own, the human cornea is unable to regenerate. The implants (marked by arrows) have stably integrated with host tissues (Figure 8.2) for over 4 years and resemble normal,

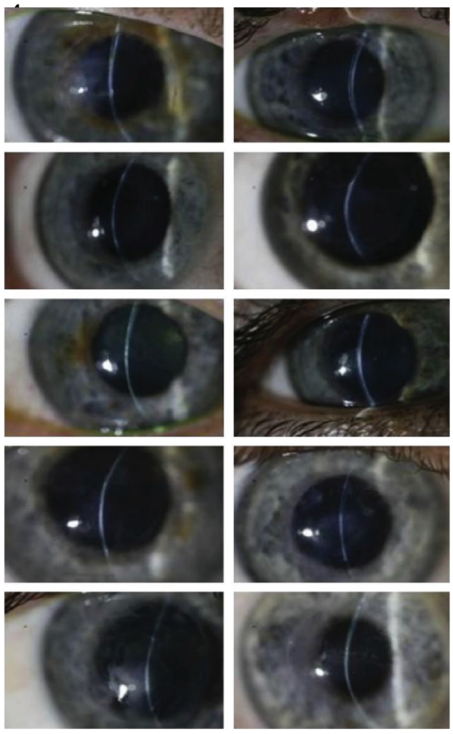


**Figure 8.2** Slit lamp biomicroscopy images of the corneas of all 10 patients at 4 years after grafting with a biosynthetic implant. Reproduced from Fagerholm et al. [6].

healthy corneas [7]. There were no signs of rejection and no need for long-term steroid immunosuppression that is required for donor transplantation. In contrast, one control donor allograft corneal implant out of 9 grafts showed rejection within the first year (11%), as per Swedish documented numbers in the cornea graft registry) [1].

However, despite this very promising, positive outcome, several issues still remained. The implants were fairly soft and the mattress sutures used to stabilize the implants sometimes left marks on the implant surface resulting in irregular astigmatism, and sometimes opacities requiring patients to use rigid contact lenses. In addition, the human cornea is avascular and needs to maintain avascularity for transparency and vision. In a rabbit alkali burn model, which recreates a severe cornea inflammation leading to immune privilege loss and neovascularization, the RHCIII implants became neovascularized [8].

It should be noted that the regeneration templates and prostheses are not mutually exclusive. Each has its own usefulness in different transplantation indications.

## 8.3 Reinforced Collagen Corneal Implants

### 8.3.1 Interpenetrating Networks of Collagen-Phosphorylcholine as Implants

To address the above issues, we reinforced the EDC/NHS crosslinked porcine collagen or RHCIII implants with a second network of (2-methacryloyloxyethyl phosphorylcholine (MPC) crosslinked with PEGDA. MPC is a synthetic phosphorylcholine lipid that is being used as anti-fouling coatings in arterial stents. Both porcine and RHCIII-MPC implants made from two interpenetrating networks (IPNs) of biopolymers were mechanically stronger, stable when exposed to enzymes [9]. RHCIII-MPC was able to resist neovascularization when tested in rabbit alkali burn models of severe pathology, where the previous generation of collagen only implants and donor allograft corneas became vascularized [8]. More recently, three patients with severe corneal pathologies (resulting from chemical burns or a previous graft rejection) were grafted with tectonic grafts of RHCIII-MPC to treat the symptoms of chronic ulceration [10]. The RHCIII-MPC implants restored the damaged stroma allowing for stable re-epithelialization and relief from pain and discomfort due to the ulceration.

### 8.3.2 RHCIII-MPC Implants in Herpes Simplex Keratitis

We implanted RHIII-MPC implants into the corneas of mice with Herpes Simplex Keratitis (HSK). Herpes simplex virus serotype 1 (HSV-1) infection of the cornea is the leading cause of infectious blindness in the developed world, and a problem in corneal grafting as there is a high risk of rejection, often due to viral reactivation. Once infected, the individuals may harbour latent virus for the rest of their lives [11].

The mouse allogeneic cornea graft model is essentially a rejection model. It allows for comparisons of time to rejection of implanted biomaterials compared to allografts. When RHCIII-MPC implants were compared to allografts in HSK mouse corneas, there was a trend towards the implants being more resistant to rejection, but the numbers were too small to show statistical significance [12].

## 8.4 Composite Corneal Implants with Peptide and Gene Therapy Capacity

### 8.4.1 LL-37

Cathelicidins are innate host defence peptides. In humans, there is only one cathelicidin, the18 kDa human cationic antimicrobial protein (hCAP18), of which LL-37 is a 37 amino acid C-terminal peptide domain with active anti-microbial and anti-viral activity [13]. In the eye, LL-37 is expressed by the cornea epithelium and has been reported to have potent anti-viral activity against Herpes Simplex Virus (HSV)-1 [14].

### 8.4.2 Implants LL-37 Peptide Release

In Lee et al. [12], we released LL-37 from silica nanoparticles (SiNPs). We showed that LL-37 was able to inhibit viral activity in cultures of human corneal epithelial cells at doses of 10–20 μg/ml, but only when the peptide was applied prophylactically (i.e., prior to viral infection of the cells). Once cells were infected with HSV-1, however, LL-37 could only delay but not prevent viral spreading to other cells. We also showed that LL-37 within SiNP showed more sustained release from collagen-MPC hydrogels (Figure 8.3) than free LL-37 alone. Encapsulation of LL-37 with alginate microparticles was not effective in preventing a burst release of the peptide, even though a high amount of LL-37 could be encapsulated. SiNP encapsulation of LL-37 and integration within a collagen hydrogel to form a composite implant

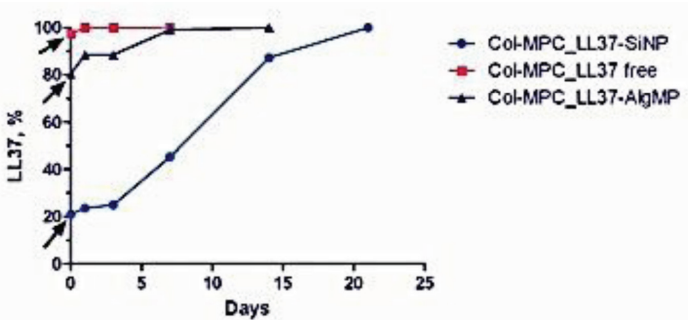



**Figure 8.3** Comparison of release profiles of free LL-37, LL-37 within silica nanoparticles (LL-37–SiNP) and alginate microparticles (LL-37–AlgMP) from within collagen-MPC hydrogels.

(Figure 8.4) therefore has the potential for providing sustained release and protection against HSV-1 infection.

It may also be possible to tether LL-37 onto or into hydrogel implants that will be used as grafts in patients with a prior history of HSV-1 infection to prevent reactivation (unpublished data). However, more research and more testing is needed to establish feasibility.

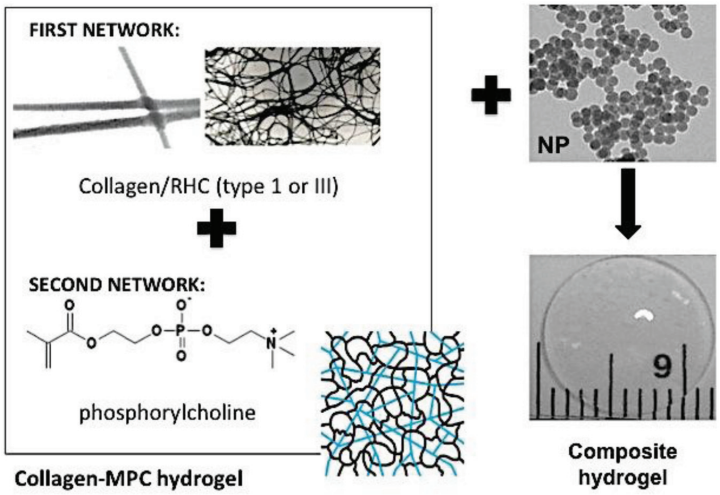



**Figure 8.4** Nano-composite, reinforced implants based on collagen-phosphorylcholine interpenetrating networks. Nanoparticles comprising silica dioxide encapsulating LL-37 peptide was evaluated for anti-HSV-1 activity.

### 8.4.3 Composite Collagen-Cell-Based Implants

We also compared the peptide treatment against gene therapy by transfecting human corneal epithelial cells with the LL-37 gene [12]. The transfected cells expressed and secreted the peptide. The secreted LL-37 inhibited viral binding *in vitro* but in this case, was insufficient to completely protect cells completely from HSV-1 infection. Nevertheless, the secreted LL-37 was able to reduce the incidence of plaque formation and reduced plaque size. The effects were overall weaker that that of exogenously applied LL-37 peptides. It is possible, however, that with further optimization of the gene transfer and copy number of transferred LL-37 genes into the cells, and/or combinations of different antiviral gene sequences, more complete viral resistance can be obtained.

In the future composite grafts releasing LL-37 or some other anti-viral compound or bioactive factor, together with genetically engineered corneal stem cells transfected with the LL-37 gene or another anti-viral gene may together stop HSV-1 activity.

## 8.5 Conclusion

We have shown that collagen-based hydrogels can be fabricated into corneal implants and used as alternatives to donor corneas for transplantation in some cases. For more severe conditions that have a higher risk of rejection of donor cornea grafts, collagen-hydrogels that are reinforced by additional interpenetrating networks of other biopolymers such as MPC, or by integration of a delivery system of drugs or other bioactives, may in the future become an alternative option to donor corneal grafting.

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## References

[1] Claesson M, Armitgae WJ, Fagerholm P, Stenevi U. Visual outcome in corneal grafts: A preliminary analysis of the Swedish Corneal Transplant Register. Br J Ophthalmol 2002; 86: 174–80.

[2] Williams KA, Esterman AJ, Bartlett, C. et al. How effective is penetrating corneal transplantation? (Factors influencing long-term outcome in multivariate analysis). Transplantation 2006; 81: 896–901.
[3] Wolfe RA. Long-term renal allograft survival: a cup half-full and half-empty. Am J Transplant 2004; 4:1215–12164.
[4] Dandona L, Naduvilath TJ, Janarthanan M et al. Survival analysis and visual outcome in a large series of corneal transplants in India. Br J Ophthalmol 1997; 81: 726–731.
[5] Iakymenko S. Forty-five years of keratoprosthesis study and application at the Filatov Institute: a retrospective analysis of 1,060 cases. Int. J. Ophthalmol. 2013; 6:375–80.
[6] Fagerholm P, Lagali NS, Merrett K, Jackson WB, Munger R, Liu Y, Polarek JW, Söderqvist M, Griffith M. (2010) A biosynthetic alternative to human donor tissue for inducing corneal regeneration: 24 month follow-up of a Phase I clinical study. Science Transl. Med. 2010; 2: 46–61.
[7] Fagerholm P, Lagali NS, Ong JA, Merrett K, Jackson WB, Polarek JW, Suuronen EJ, Liu Y, Brunette I, Griffith M. (2014) Stable corneal regeneration four years after implantation of a cell-free recombinant human collagen scaffold. Biomaterials 2014; 35: 2420–2427.
[8] Hackett JM, Lagali N, Merrett K, Edelhauser H, Sun Y, Gan L, Griffith M and Fagerholm P. Biosynthetic corneal implants for replacement of pathologic corneal tissue: performance in a controlled rabbit alkali burn model. Invest Ophthalmol Vis Sci 2011; 52: 651–657.
[9] Liu W, Deng C, McLaughlin CR, Fagerholm P, Watsky MA, Heyne B, Scaiano JC, Lagali NS, Munger R, Li F, Griffith M. Collagen-phosphorylcholine interpenetrating network hydrogels as corneal substitutes. Biomaterials 2009; 30: 1551–1559.
[10] Buznyk O, Pasyechnikova N, Islam MM, Iakymenko S, Fagerholm P and Griffith M (2015) Bioengineered Corneas Grafted as Alternatives to Human Donor Corneas in Three High Risk Patients. Clin Transl Sci 8: 558–562.
[11] Choudhary A, Higgins GT, Kaye SB. Herpes Simplex Keratitis and Related Syndromes. In: Reinhard T, Larkin F, eds. Cornea and External Eye Disease: Springer Berlin Heidelberg; 2008:115–152.
[12] Lee CJ, Buznyk O, Kuffova L, Rajendran V, Forrester JV, Phopase J, Islam MM, Skog MM, Ahlqvist J and Griffith, M. (2014) Cathelicidin LL-37 and HSV-1 corneal infection: Peptide versus gene therapy. Transl Vis Sci Technol 2014; 3: 4.

[13] Dürr UHN, Sudheendra US, Ramamoorthy A. LL-37, the only human member of the cathelicidin family of antimicrobial peptides. Biochimica et Biophysica Acta (BBA) – Biomembranes 2006; 1758:1408–1425.
[14] Gordon YJ, Huang LC, Romanowski EG, Yates KA, Proske RJ, McDermott AM. Human cathelicidin (LL-37), a multifunctional peptide, is expressed by ocular surface epithelia and has potent antibacterial and antiviral activity. *Curr Eye Res.* 2005; 30:385–394.