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Abstract

Purpose of review: Artificial corneas are being developed to meet a shortage of donor corneas as well as to address cases where allografting is contraindicated. A range of artificial corneas has been developed. Here we review several newer designs and especially those inspired by naturally occurring biomaterials found with the human body and elsewhere.

Recent findings: Recent trends in the development of artificial corneas indicate a move towards the use of materials derived from native sources including decellularized corneal tissue and tissue substitutes synthesized by corneal cells *in vitro* when grown either on their own, or in conjunction with novel protein-based scaffolds. Biologically inspired materials are also being considered for implantation on their own with the view to promoting endogenous corneal tissue.

Summary: More recent attempts at making artificial corneas have taken a more nature-based or nature-inspired approach. Several will in the near future be likely to be available clinically.

Keywords: biomaterials, cornea, transplantation, clinical observations

Introduction

According to the World Health Organisation, there are 285 million visually impaired individuals worldwide, of whom 39 million are blind [1]. Corneal blindness is the fourth largest cause of blindness, making up 5.1% of cases with an estimated 1.5 to 2.0 million new cases of unilateral blindness being reported annually [2]. While many of these patients can be treated by receiving a donor corneal tissue transplant, donor corneas are in limited supply globally and a high proportion of allografts, given sufficient time, will eventually fail due to immunological factors. In addition, there are instances where the use of donor tissue is contraindicated. For these reasons there has long been interest in developing alternatives to donor corneas as materials for restoring corneal tissue structure and function.

A number of prosthetic corneas or so-called keratoprostheses (KPros) are currently used clinically and improvements in design since first conception have led to acceptable performance. Examples include the Boston KPro [3], AlphaCor [4] and the osteo-odonto-keratoprosthesis or OOKP [5]. Significantly, two of these examples make use of natural materials to achieve a biocompatible host-graft interface. The Boston KPro uses a cross-linked human donor cornea rim as the interface [6], while the OOKP contains dental tissue wrapped with autologous oral mucosal cells.

More recently, the use of decellularized extracellular matrices (ECM) of various target organs have been proposed as scaffolds for regeneration. For example, a recent news article reports a clinical trial of 115 Chinese patients who were transplanted with decellularized porcine corneas [7]. The report states that “Seventy percent of the replaced corneas are clear”. These are technically xenografts and hence fall outside the scope of this review. As an alternative to solid organ

transplants (either human or animal) some groups have used corneal cells to construct their own ECM *in vitro*, while others have used ECM macromolecules and mimics to develop artificial corneas that induce endogenous cells from the host to migrate into the implant to affect the repair. There are various reviews on the different artificial corneas and their performance [8-11]. Here, we review several of the artificial corneas that are inspired by nature and natural ECM materials, and discuss in detail the creation of tissue templates designed to promote corneal tissue regeneration following transplantation as opposed to solid organ transplants.

The Original Artificial Corneas

The term "artificial cornea" strictly speaking refers to keratoprostheses that comprise plastic polymers and other synthetic materials. However, more recently, this term has come to be used more broadly (albeit perhaps inappropriately) to refer to any material implanted with the view to to restoring the full structure and function of the cornea. Early KPros such as the Boston KPro, OOKP and AlphaCor have been helping individuals save their vision, when allografting was contraindicated. Newer iterations of KPros developed by Storsberg [12] and Myung [13] are using sophisticated surface coating and patterning techniques to obtain an optimal host-graft interface, and these are being tested clinically [12].

Artificial Corneas Fabricated from Biological Materials

The ECM of any organ is composed of a range of both structural as well as instructional macromolecules. During embryonic development, primordial ECMs contribute to organogenesis by providing cues for the cells to convert the organ anlagen into a fully formed and functional organ [14]. In wound healing the same ECM macromolecules contribute to tissue repair and

regeneration, and therefore can be considered as ideal components for fabricating implants that would enable regeneration. Several newer “artificial corneas” that have been tissue engineered are therefore designed with the use of ECM components or polymers that mimic the ECM in its function.

- **Corneas from Self-Assembly**

One ingenious method for using ECM macromolecules is to coax the cells of the target organ to produce their own ECM. Germain and co-workers have developed a range of “self assembled” human corneal equivalents. Their latest model uses all three corneal cell types [15]. They start by creating the stroma by culturing stromal fibroblasts in growth medium supplemented with ascorbic acid for 28–35 days. The ascorbic acid stimulates the fibroblasts to secrete extracellular matrix components such as collagen, forming sheets of 35-55 microns in thickness. These are then stacked together to form a corneal stroma. Corneal endothelial cells were then seeded on top of this stacked stroma and allowed to form a monolayer in endothelial growth medium. After two to seven days, a plastic ring is placed on top of the cultured construct and this is flipped upside down. Corneal limbal epithelial cells are then seeded on top and the entire construct is cultured in epithelial cell medium supplemented with more ascorbic acid. After another 7 days in culture to enable epithelial cell confluence, the entire construct is air-lifted and cultured at the air-liquid interphase to promote for stratification of the corneal epithelium. The resulting corneal construct as reported in Proulx et al. [15] was fairly transparent. However, it was only a fraction of the thickness of a human cornea. The authors suggest, however, that the required thickness can be readily achieved by simply increasing the number of stacked layers of fibroblast/ECM. There is no available information in the literature of clinical testing.

More recently, Karamichos et al. [16] reported the use of ascorbic acid (with and without additional transforming growth factor-beta) to induce secretion of ECM by human umbilical cord mesenchymal stem cells (cord stem cells) to form a cornea stroma-like construct. In comparison to human cornea fibroblasts, the cord stem cells produced a more extensive matrix. In addition, the cells themselves differentiated into stroma-like cells. However, despite the ability of the umbilical cord stem cells to produce a stroma, it took four weeks to produce a construct that was 24 mm in diameter and 30 microns thick.

A main drawback, therefore, is the long period of time required for the fabrication of a construct. The use of allogeneic cells might also be problematic, so implants fabricated in this manner will most likely be confined to autografts in patients in carefully pre-planned surgeries, as opposed to being an off-the-shelf solution. Nevertheless, self-assembled corneal stromal constructs could be decellularized and used as implants. This possibility has not been reported for the cornea but has been reported for tissue engineered cartilage [17].

- **Collagen Based Constructs**

As collagen is the main structural component of the ECM, there has been a lot of interest in collagen-based implants. The main source of collagen is extracted animal protein, although marine collagen is now being used for tissue engineering [18]. Recent developments in the area include the use of plastically compressed collagen that was compressed with [19] and without pre-seeded keratocytes [20]. Compressed collagen as stromal substituted have now been tested in rabbits as implants. The implants were translucent, but non-immunogenic and supported epithelial overgrowth [19]. Vitrified collagen has also been used to fabricate membranes with good mechanical strength for corneal repair [21].

- **Silk Fibroin Based Constructs**

While collagen is an obvious candidate for corneal reconstruction, materials of non-animal origin have also been explored, primarily in an effort to reduce production costs and limit potential exposure to infectious materials. One such material that has recently been explored in some detail, is the structural protein found within silkworm-derived silk fibers known as fibroin [22]. In particular, given its relatively safe history of prior clinical use (i.e. in the form of silk sutures), the majority of studies have been based upon fibroin produced by the domesticated silkworm *Bombyx mori*.

Aqueous solutions of peptide fragments derived from *B. mori* silk fibroin (BMSF) can be readily produced from whole cocoons using relatively simple and inexpensive processing techniques [23]. While little of the native full-length protein remains following processing, the resulting peptide solutions can subsequently be used to generate a variety of stable and biocompatible materials including freestanding membranes, electro-spun fibers and porous sponges [24]. Of these three materials, the freestanding BMSF membranes have received most attention for potential use in corneal tissue reconstruction owing to their high degree of transparency and general handling properties [25-28]. Importantly, subsequent studies have revealed that BMSF-based materials support the attachment, growth and differentiation of cells isolated from all three main tissue layers of the cornea [25, 28-30]. These results are in themselves quite remarkable given that BMSF, unlike collagen, does not possess specific structural motifs designed to promote cell attachment and growth. Nevertheless, a sufficient level of cell attachment can be achieved through use of serum-supplemented culture medium and the opportunity to customize cell attachment is supported through coating with either purified ECM components [30, 31] or peptides of choice [32]. Based upon these findings, one of our groups (DGH) is currently

engaged in preclinical studies of BMSF as a vehicle for delivering cells for corneal reconstruction. For example, BMSF membranes are being investigated for their potential to deliver limbal epithelial cell cultures to the ocular surface [28] and to implant corneal endothelial cells cultures to the posterior surface [30]. In addition, Lawrence et al. [26] have proposed using stacked layers of stromal cells grown on BMSF-membranes to repair the corneal stroma. Theoretically, combining these approaches could lead to creation of a full-thickness corneal tissue substitute as described in Figure 1. Preliminary studies by Higa et al. [27] indicate that single BMSF-membranes are well tolerated when implanted within the corneal stroma, but safety and efficacy data for of any combination of cells grown on BMSF membranes has yet to be reported.

Artificial Corneas from Synthetically Produced Biologically Inspired Materials

Although extracted ECM macromolecules have now been used for tissue engineering, the extracted materials are often difficult to purify and are subject to batch-to-batch variation. There is a small but present risk of disease transmission from extracted animal products. From a tissue engineering perspective, native ECM macromolecules are often large, complex macromolecules and difficult to manipulate, as they lack suitable functional groups for chemical modification.

- **Self-Assembling Peptides as Corneal Scaffolds**

The ECM is made up of a range of biological polymers, which have self assembled. Smaller units of these proteins, sugars and lipids have therefore been tested for their ability to function as more controllable mimics of the ECM [33]. Of these, the peptides have been most extensively examined as they can form a wide range of structures including nanofibres that mimic the ECM.

There are several ways to design self-assembling peptides (SAPs) that are being evaluated for regenerative medicine. Ionically complementary peptides, which were first described in 1993 by Zhang and co workers, comprise short peptide repeats, with hydrophilic and hydrophobic "faces" that stack to form long, entangled fibres [34]. Peptide amphiphiles, comprise hydrophilic peptide sequences that are attached to a hydrophobic tail.

Connon et al. [35] designed two synthetic peptide amphiphiles that contain the well-known cell adhesion motif from fibronectin RGD. These SAPs were then assembled into film coatings that were able 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. Uzunalli et al. [36] incorporated laminin motifs into peptide amphiphiles that were developed as injectable scaffolds, and tested them in rabbit models of wound healing. They reported an increase in keratocyte migration into the surgically induced wound, resulting in enhanced stromal regeneration. The use of self-assembled peptides is an exciting and promising new technology, and in the near future, corneal implants fabricated from SAPs will likely be appearing in the literature.

- **Recombinant Human Collagen-Based Corneal Implants**

In contrast to the short self-assembling peptides, the recombinant collagens that have been produced and are commercially available, reproduce full-length collagen fibrils with the same amino acid sequence as human collagen. By co-expression of prolyl hydroxylase genes with the collagen genes, recombinant collagens with a similar degree of stability as naturally occurring material can be produced. Like native type I and III structural collagens, the recombinantly produced counterparts also assemble into their characteristic triple helices.

Type III recombinant human collagen (RHCIII) has now been fabricated into corneal implants and have tested in a small Phase I clinical study by one of us (MG) as possible substitutes for donor corneas. These 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) cross-linked RHCIII constructs, which were designed as simple mimics of the corneal stromal ECM, were implanted into the corneas of 10 patients (nine with keratoconus and 1 with a central scar) in Sweden by anterior lamellar keratoplasty [37]. These cell-free implants induced the patient's own epithelial cells to grow over the implants, while stromal cells migrated into the implants, anchoring them stably into the eye. The in-growth and/or proliferation of stromal cells actively continued over the four-year observation period [38]. Corneal nerves also regenerated over the four years. The regenerated neo-corneas remained seamlessly and stably integrated in the operated eyes (Figure 2) without any immunosuppression beyond a short course of prophylactic steroids, in comparison to the longitudinally followed donor cornea patients who received 12 months of steroids. There was no rejection in implanted patients while one patient out of the 9 donor grafted individuals (11%) had a rejection episode in keeping with the current statistics of 10-15% rejection within the first year of grafting [39]. There was no recruitment of inflammatory dendritic cells into the region of the implant. In contrast, patients transplanted with donor corneas showed dendritic cell migration into their central cornea. One patient underwent regrafting even though he had a clear and stably integrated cornea, since contact lenses required for good visual acuity could not be fitted. Significantly, the excised corneal button displayed a normal corneal architecture when examined by histology (Figure 3). Observations using *in vivo* confocal microscopy revealed that donor human cornea grafted eyes had abnormally tortuous nerves and stromal cell death was found. Regenerated neo-corneas displayed similar nerve morphology of parallel bundles although the numbers were significantly fewer than in normal

healthy corneas. The implanted patients had a 4-year average corrected visual acuity of 20/54 and gained more than 5 Snellen lines of vision on an eye chart. This visual acuity can be improved with the use of more robust materials for better shape retention or a different suture retention method that does not impede epithelial re-grow into the centre of the cornea. Despite these remaining issues, RHC implants can nevertheless achieve stable regeneration and therefore, represent a potentially safe alternative to donor organ transplantation.

Conclusions

Despite being considered as arguably one of the most successful types of solid organ transplants, the limited supply and health risks associated with donor corneas have driven the quest for alternative tissue substitutes. While earlier strategies focused on the use of synthetic materials, recent iterations of these technologies have exploited incorporation of natural tissue, especially with respect to enabling better bio-integration. Later designs, however, are now actively exploiting the potential of naturally occurring biomaterials such as collagens and silk fibroin as scaffolds for tissue reconstruction and/or delivery of cell-based therapies. Furthermore, recent progress in the design and production of synthetic self-assembling peptides, seems likely to pave the way for a new generation of corneal tissue substitutes which when used in conjunction with co-emergent technologies (e.g. induced pluripotent cells stems) might soon be tailored to meet the needs of individual patients.

Key points:

- Artificial corneas based on naturally occurring biomaterials and structural/functional mimics have now been developed.

- The design and development of a selection of these biological based or inspired implants are discussed.
- Highlights include the use of silk based material as a potential scaffolds for corneal stem cell grafting, and the long-term clinical trial results showing stable regeneration of corneal tissue and nerves without immunosuppression after implantation with recombinant human collagen-based artificial corneas.

Conflicts of interest: None

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Figures

Figure 1. Schema of proposed strategy for fabricating an artificial corneal transplant using silk fibroin membranes in conjunction with cultured cells (modified from a similar figure illustrated by Bray *et al* [23], according to a strategy proposed by Harkin *et al* [24] and Lawrence *et al* [26]). Conceptually, the required number of transparent fibroin membranes with cultured stromal cell layers (ideally maintained as keratocytes) could be combined with upper and lower layers composed of cultured limbal epithelial cells and corneal endothelial cells respectively grown on fibroin membranes. All three types of cells display acceptable growth on fibroin [20-25] and the membranes themselves are well tolerated when implanted into the corneal stroma of rabbits [22]. *In vivo* studies of the proposed construct have yet to be reported.

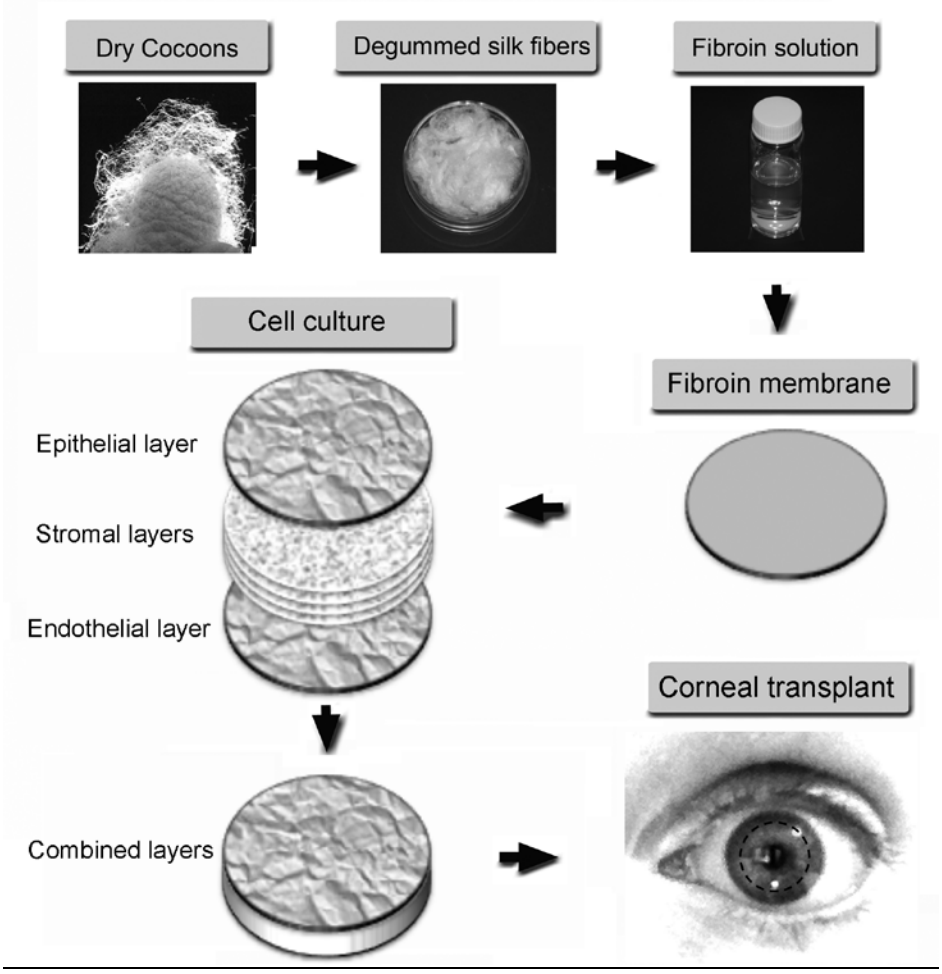

Figure 2. Slit lamp biomicroscopy images of the regenerated corneas of all 10 patients at 4 years after grafting with a cell-free implant made from EDC/NHS crosslinked recombinant human collagen. Reproduced from Fagerholm et al. Biomaterials 35: 2420-2427, 2014 [33].

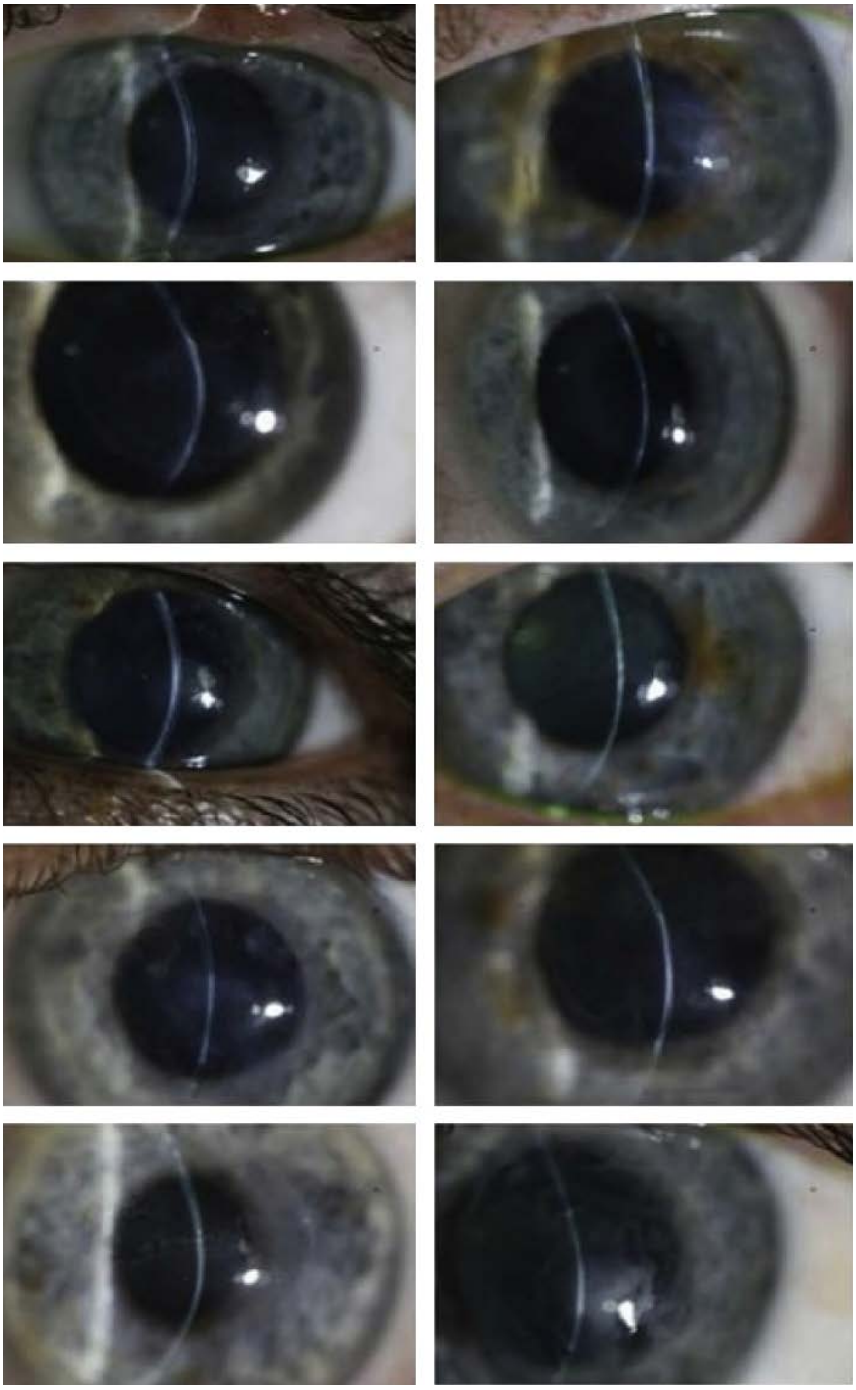

Figure 3. (A) Histological section through a regenerated neo-cornea that was obtained after 4 years post-operation, when the patient was re-grafted, showing a normal corneal morphology. with stratified epithelium (ep), lamellarly arranged stroma (s) and endothelium (en) that was left intact during the implantation. Part of the recombinant human collagen implant (i) is still present, showing a slow but active remodeling process over time. (B) Higher magnification showing an area of the cornea where remodeling is more advanced and the implant (i) is seamlessly blending into the stroma (s). ep, epithelium. Scale bars, 100 µm. Reproduced from Fagerholm et al. Biomaterials 35: 2420-2427, 2014 [33].