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Biologic Keratoprosthesis Materials

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The keratoprosthesis (KPro; “artificial cornea”) offers patients with cornea blindness, the hope of visual recovery. The first KPro designs entailed placing an optically clear material through an opaque cornea. Although introduced over 200 years ago, the basic concept is retained in many of the modern KPro designs. The Boston KPro, employs a polymethylmethacrylate (PMMA) (inert plastic) optic stabilized between a front and back plate. With over 2000 implanted world-wide to date, the Boston KPro has an excellent retention rate in patients who have failed prior penetrating keratoplasties, but lack chemical injuries or auto-immune cicatricial diseases, such as Stevens-Johnson syndrome or pemphigoid. From the Boston KPro multicenter study, the patient population who had a primary diagnosis of graft rejection retained the KPro in 97% of the eyes with a mean follow-up of 8.5 months.¹ However, patients with cicatricial autoimmune disease had a retention rate of 83% and those with chemical injuries as a primary diagnosis had an 89% retention rate. Over a longer follow-up period, the retention rate decreases further for patients with autoimmune diseases and chemical injuries.^{2,3} For this subset of desperate patients, we continue to search for methods of increasing the retention rate of a KPro.

Some KPro models differ from early designs by surrounding a central optic with a carrier or haptic that is intended to “biointegrate” with the cornea. In theory, the KPro could potentially integrate with the cornea epithelium, stroma, endothelium, or a combination of the corneal layers. Most of the “biointegratable” KPro research has focused on integration with either the cornea epithelium or the stromal keratocytes. Integration with the cornea epithelium may offer the advantage of a barrier to infection, but it would offer little structural support. Given that the stroma comprises about 90% of the cornea thickness, a type of stromal adhesion may increase the structural integrity of a KPro.

Bio-integratable KPro designs can be separated into autologous biologic models and other models derived from biologic materials. These models include biologic haptics, coatings, and scaffolds. This paper will review both the clinical use of and the research on biologic KPro models.

Autologous Biologic Haptics

Autologous biologic models use a central optic supported by a skirt of either tibia bone or the root of a tooth with its surrounding alveolar bone. Because allogeneic, in contrast to autologous, biologic models require systemic immune suppression and their use can have associated complications, its use has been mostly abandoned. The osteo-odonto KPro

(OOKP) use was first described in the 1960s.^{4,5} A PMMA optical cylinder is glued to an osteodental lamina derived from an extracted tooth root and attached alveolar bone which has been removed from the patient's jaw. The OOKP is then implanted into a cutaneous infraorbital pouch, where it is left in place for a few months. The OOKP is then removed from the infraorbital pouch and implanted into the eye. After the diseased cornea has been trephined and removed, the OOKP is sutured to the sclera. Finally, a mucous membrane graft covers all but the central optical center. The eye reportedly has good bio-integration into the osteodental lamina with the deposition of extracellular matrix of elastic/elastin fibers and collagen.⁶ Bone remodeling also continues to occur. Histologic examination by Pecorella found increased bone deposition and ossification of the dental and alveolar bone surfaces and absence of diffuse bone absorption. However, 3-dimensional computed tomography imaging found varying levels of bone absorption without a loss of stability of the OOKP complex. All of the 9 eyes studied demonstrated a minor reduction of the lamina. One patient demonstrated complete absorption of the inferior osteodental lamina.⁷ However, good long-term anatomic and functional outcomes have been reported from 181 eyes.⁸ The study found that the OOKP had a very good anatomic retention rate of 94% after a median follow-up of 12 years. Their mean best postoperative acuity was 0.76 (logMAR). Similarly, very good retention rates were found in studies by Hille et al (25 of 25, 100%, 3 y median follow-up)⁹ and by Marchi et al (83 of 85, 97.5%, minimum follow-up of 10 y).¹⁰ However, in some of the reports the preoperative categories were not specifically identified.

A small piece of the patient's tibia can also be used as a carrier for an optical core.^{11,12} A small chip is removed from the patient's anterior tibia and then fashioned into a disk about 4 mm in size. A central core is removed and, like the OOKP, a central PMMA cylinder is glued in place to produce the tibia bone KPro (TKPro).

A study comparing different types of KPro demonstrated increased absorption of the TKPro's tibia bone compared with OOKP's dentin lamina.⁹ Of the 4 eyes implanted with the TKPro, Hille et al reports massive bone absorption in 1 (25%) of the eyes requiring removal after 1 year. Conversely, only 2 of 25 (8%) OOKP eyes experienced bone absorption. Mucous membrane defects leading to exposure of the underlying alveolar bone occurred perioperatively in 10 (40%) of the OOKPs and in 1 of the TKPros. Mucus membrane defects can potentially lead to cornea perforations or KPro extrusions. Temprano¹² reported an extrusion rate of 25% in his TKPros in his study, which included 167 implants. Falcinelli et al noted cornea perforations in 5 of 181 (2.7%) of his OOKPs.

Both of the autologous biologic KPro designs reportedly have good biocompatibility and biointegration but the popularity of these designs is limited by several factors. Similar to other KPro models, the visual potential from tooth and bone KPros is often limited by glaucoma, retroprosthetic membranes, and retinal diseases.^{8,9} Loss of the overlying oral mucus can result in corneal perforations or extrusions. Unlike other types of KPros, the autologous biologic KPros are surgically more invasive, requiring 1 or 2 surgical procedures to nonocular sites. This may require a coordinated surgical procedure with a nonophthalmologist. The OOKP requires a healthy tooth and jaw. One third of the patients in Hille et al's study lacked a suitable tooth for the procedure.⁹ In addition, both the TKPro and the OOKP are staged procedures, necessitating a return to the operating room and a

delay in any visual rehabilitation. These factors may limit the number of patients and surgeons who are eager to embark on an experience with either the TKPro or the OOKP, despite their biointegration, anatomic retention, or their functional success.

Hydroxyapatite Biologic Haptics

The eye's successful biointegration with the tooth or bone served as the inspiration for biologic coatings and their materials to be used as a haptic for a KPro. Hydroxyapatite has a similar mineral composition to both bone and teeth. Made of phosphate and calcium, hydroxyapatite is frequently used as a bone substitute and as a bioactive prosthesis within the orbit.¹³ It is also thought that hydroxyapatite causes minimal tissue inflammation, is not absorbed, and promotes tissue in-growth.¹⁴ These principles serve as the framework behind the Coralline hydroxyapatite KPro (HAKPro).¹⁵ Using a coral skirt stabilizing a central optic, the HAKPros were first studied in the eyes of 12 New Zealand rabbits. The investigators used 2 different types of Caribbean coral and half of the HAKPro were covered by a conjunctival flap. After various postoperative periods (2, 5, 7, and 9 mo), all of the eyes sent for histopathologic studies demonstrated fibrovascular tissue in-growth. Technetium-99 bone scans showed uptake of radioactive material by the vascularized hydroxyapatite implants. No differences were seen between the 2 types of coral. The group of HAKPros that lacked a conjunctival flap all developed small inferotemporal melts or erosions requiring further surgical repair. Bone differentiation was also observed. Osteoblasts and osseous formation were seen on the surface of some of the hydroxyapatite. Overall, the HAKPros demonstrated good biocompatibility and were colonizable.

Hydroxyapatite was also found to induce superior keratocyte proliferation and adhesion in vitro when compared with 3 other materials currently used as a KPro.¹⁶ Mehta et al studied the adhesion and growth of human keratocytes in vitro to hydroxyapatite, glass, polytetrafluoroethylene, and polyhydroxyethylmethacrylate. Polytetra-fluoroethylene, otherwise known as Teflon, is the material used as a skirt in the Legeais BioKPro III. The AlphaCor device uses hydroxyethyl-methacrylate, a hydrogel, as a skirt supporting a central optical core. On the hydroxyapatite surfaces, superior keratocyte adhesion was demonstrated by increased focal adhesions (vinculin immunostaining) and increased expression of $\beta 1$ -integrin when compared with the other materials.

Hoping to preserve hydroxyapatite's biocompatibility but to eliminate its brittle, rigid texture, researchers from China combined a porous nano-hydroxyapatite with a hydrogel to create a biologic haptic for a KPro.¹⁷ The pores, measuring 80 μm , within the composite hydrogel were designed to allow for the free diffusion of water, glucose, and nutrients. Poly (vinyl alcohol) combined with hydroxyapatite was compared with pure polyvinyl alcohol after their insertion into intrastromal pockets in 10 rabbits. After 1 month, the skirt material appeared to be vascularized by slit-lamp examination. There was no evidence of intrastromal inflammation of the abutting cornea or encapsulation of the skirt material. Histologic studies demonstrated host tissue in-growth with the deposition of collagen and blood vessels within the hydrogel pores. The paper did not comment on any complications in the long-term post-operative period. The investigators noted that neither inflammatory cells nor epithelial cells adhered to the skirt material. Although the visual potential of the device was not addressed

within the paper, postoperative photographs show superficial vessels and fibrotic tissue growing over both the skirt and the optical center of the KPro. As an intralamellar device embedded within the cornea, this model of KPro would be unlikely to provide visual benefit to eyes with anterior cornea disease or cornea neovascularization.

Collagen Biologic Coatings and Scaffolds

While research on hydroxyapatite has focused mostly on the cornea stromal cells, much of the scientific work on biologic coatings has tried to improve the growth and biocompatibility of cornea epithelial cells. Because of their desmosome and hemidesmosome tight junctions, epithelial cells are the body's first line of defense as a barrier to infection. Desmosomes connect the epithelial cells to each other, whereas hemi-desmosome are formed between the epithelial cells and extracellular material, such as a basement membrane. Ideally, a KPro would be anchored to the cornea epithelium by hemidesmosomes, thus creating a barrier to infection. A KPro with a biologic coating may be able to achieve this enormous feat.

Some basic knowledge of the process of cornea epithelial wound healing is helpful to understand why researchers have chosen various biologic coatings. Cornea epithelial wound healing can be broken down into 3 stages: cell migration, proliferation, and adhesion.^{18,19} Cornea epithelial cell migration is the first step and is initiated after the recognition of extracellular matrices by epithelial adhesion receptors.²⁰ Fibronectin, collagens, vitronectin, and laminins constitute much of the extracellular matrix of the healing cornea epithelium basement membrane. Previous research has shown that fibronectin is not detected in normal rabbit cornea, but does appear below migrating epithelial cells during wound healing.²¹ The basement membrane of the cornea epithelium is composed of mostly type IV collagen, laminin, and heparin. Also, one of the components of the basement membrane, laminin (specifically laminin V) is a pivotal part of the of the hemidesmosomal complex.²² It also seems to play an important role in wound healing. Studies have shown that there is a correlation between the early appearance of laminin and hemidesmosome formation.^{23,24} By coating synthetic materials with bio-proteins, such as collagen, fibronectin, and laminin, researchers hope to improve epithelial cell adhesion and proliferation to implant surfaces.

The effect of biologic coatings on epithelial growth and attachment to synthetic polymers has been evaluated by several researchers. The cornea epithelium's growth on polymers with various surface properties was measured quantitatively in vitro by Pettit and colleagues.²⁵ They found better epithelial cell growth on polymers that were coated with fibronectin than those coated with IgG or albumin. Similarly, Ohji et al²⁶ also found that fibronectin coating increased epithelial cell growth in vitro when compared with controls. Using human corneal epithelial cell seeded onto coated culture plates, the study quantitatively measured the cellular attachment to plates coated with laminin and fibronectin. Although both proteins increased the attachment of cells when compared with uncoated controls, laminin demonstrated the greatest percent increase in cellular attachments to the plates.

While attempting to develop a successful material for a synthetic cornea onlay, Sweeney et al²⁷ experimented with various biologic coatings. The research hoped to identify a coating that would promote the best epithelialization over a porous polycarbonate membrane. The in

vivo study in cats also evaluated laminin and fibronectin as well as the following surface coatings: collagen I, collagen III, collagen IV, chondroitin sulfate, and hyaluronic acid. The eyes were evaluated by immunohistochemistry and electron microscopy at various time points, up to 10 weeks, after implantation. Collagen I, collagen IV, and laminin supported consistent multilayered epithelialization of the synthetic implant. All of these coatings also demonstrated attachment of corneal epithelial cells to the implant surface. Histologically, a band of extra-cellular matrix, similar to a basement membrane, was seen between the epithelium and the implant. Furthermore, hemidesmosomal adhesion plaques were visible between the epithelial cells and the polymer implant. Overall, collagen I performed best with the greatest amount of stratification and differentiation of the epithelial cells. The authors recommended using collagen type I as a coating for a synthetic onlay.

Collagen has also been investigated as a coating for polymers and also as part of the architecture of synthetic polymers. Researchers from Australia investigated the adherence of epithelial cells to polymers that were coated in collagen.²⁸ A cross-linked collagen I gel coating was compared with a simpler form of covalent coating of collagen on a porous synthetic polymer in vitro. The investigators found hemidesmosomal plaque formations that were interrupted by the pores. In addition, extracellular matrix material was deposited on the polymer surface and within the pores of the uncoated and the covalently coated collagen surfaces. In comparison, the polymer coated with cross-linked collagen gel demonstrated a more continuous pattern of hemidesmosomal plaques, a primitive basal lamina, epithelial bridging of the pore openings, and anchoring fibril formation between the epithelial cell membranes and the polymer surface. Therefore, the authors proposed that the cross-linked collagen gel was perhaps better recognized by the epithelial cells, which allowed them to respond by assembling more intricate adhesive structures.

Researchers from Canada and Singapore have also taken advantage of the biocompatibility of type I collagen and combined it with glycoaminoglycans (GAGs) to construct synthetic stromal scaffolds.^{29–34} The team from Singapore fashioned a nanofibrous collagen-GAG scaffold by electrospinning the collagen blended with chondroitin sulfate, a GAG, into uniform fibers.³⁵ After cross-linking the scaffold with glutaraldehyde vapor, the synthetic cornea supported in vitro rabbit conjunctival fibroblast growth without degradation. Although cornea cell lines have yet to be studied, it was concluded that the scaffold demonstrated good biocompatibility because the small size and uniform structure of nano-scale fibers mimicked that of the natural cornea. Griffith and researchers²⁹ from Canada describes a stromal scaffold that was also formed from collagen that had been glutaraldehyde cross-linked to slow the degradation from cornea collagenases. They further experimented with other cross-linking methods such as γ irradiation, urethane, dialdehyde, and carbodiimide-based cross-linking. The same research team from Canada also studied the incorporation of YIGSR, a laminin-based peptide, into a carbodiimide collagen cross-linked gel. They found both in vitro epithelial cell growth onto and nerve growth into the YIGSR-modified collagen gels.³¹ After 1 year in porcine eyes, they found cornea nerve regeneration and growth in all layers of the synthetic cornea similar to control eyes.³² In mice using porcine collagen, similar tissue engineered corneas were implanted and eventually led to the development of retroprosthetic membranes associated with a significant macrophage and neutrophil response.³³ The retro-prosthetic membranes were developed 7 to 10 days

postoperatively. Although synthetic collagen-based cornea scaffolds still have some barriers to overcome, both the research from Canada and Singapore shows much promise with regard to biointegration and biocompatibility of the tissue-engineered cornea.

Conclusions

Although the concept of an artificial cornea is over 200 years old, the research and clinical use of the KPro has recently increased. Models using biologic materials vary and show different levels of promise. The OOKP and its tibial equivalent have reportedly enjoyed very good biointegration and retention rates. Their widespread use may be ultimately limited by the invasive nature of the procedures needed to implant the devices. Research into less invasive means of incorporating hydroxyapatite, the core component of the OOKP, has shown favorable results. Coating potential KPro designs with biologic materials, such as collagen, has also demonstrated promise. The cornea's biocompatibility with collagen should not be surprising given the fact that it comprises most of the native cornea tissue. Henceforth, the collagen-based scaffolds resemble the natural cornea's structure and seem to have much potential. Although the perfect KPro has yet to be developed, the current research using biologic materials is promising and may eventually offer sight to the millions of people impaired by corneal blindness around the world.

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