Tissue Engineering in Ophthalmology: Implications for Eyelid Reconstruction

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Purpose: Bioengineering aims to produce functional tissue replacements to repair defects and has been widely investigated over the past few decades. We aimed to review the available literature on the application of tissue engineering in ophthalmology, with a particular focus on ophthalmic plastic surgery and potential applications for eyelid reconstruction.

Methods: A literature search was performed on the MEDLINE database using the keywords "bioengineering," "tissue engineering," and "ophthalmology." Articles written in English were included.

Results: There is a substantial body of work on tissue engineering of the cornea. Other structures in ophthalmology investigated include the conjunctiva, lacrimal gland, and orbital bone. We also discuss the potential application of tissue engineering in eyelid reconstruction.

Conclusion: Tissue engineering represents the future of regenerative and reconstructive medicine, with significant potential applications in ophthalmic plastic surgery.

Bioengineering represents the future of reconstructive medi- cine, but there are few studies that investigate the role of bioengineering in ophthalmic plastic surgery. We have sum- marized the key components of bioengineering below, with a focus on cellular scaffolds for tissue replacement. We review the available literature on its application in ophthalmology includ- ing corneal replacement, conjunctival repair, dry eye disease, and orbital fracture repair and focus on the potential applica- tions in the eyelid.

Tissue engineering refers to the synthesis of living tis- sues using bioreactors, cells, scaffolds, and/or growth factors with the aim of creating a functional tissue replacement to repair defects. The use of engineered 3-dimensional (3D) biomaterial constructs to reconstruct or repair living tissue has been widely investigated over the last 2 decades.[1–3] Ideally, bioengineered tissue would restore key functions of missing or defective tis- sues and would degrade at a rate which best complements the natural rate of cellular differentiation and proliferation,

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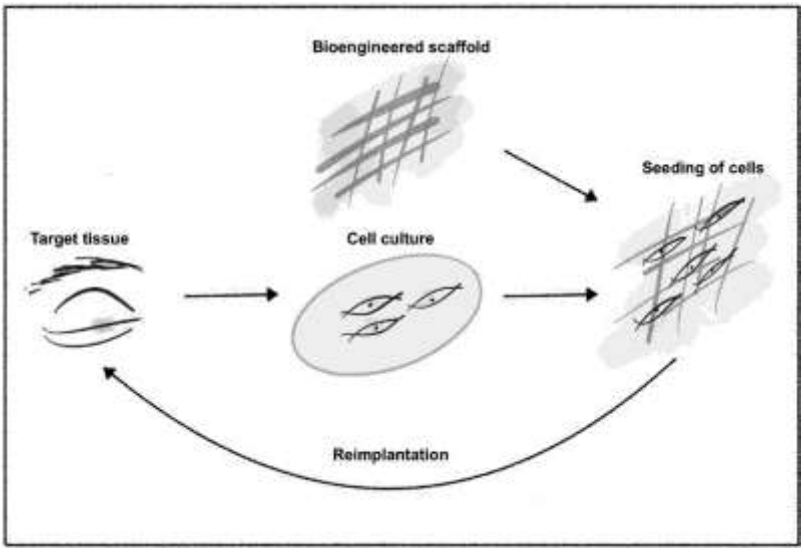
ultimately integrating well with surrounding native tissue both in the immediate and long-term period. The basic principle of tissue engineering generally involves the combination of a poly- mer scaffold with a stem cell or precursor cell population. Key components required for successful tissue engineering include a viable scaffold, cells, stimulating factors to encourage desired cell behavior, and a blood supply. Figure demonstrates the basic principles of tissue engineering.

The use of a porous scaffold to provide support and facilitate synthesis of 3D tissue represents

one of the principal methods of tissue engineering. The role of the scaffold includes supporting and guiding cell attachment and tissue growth, pro- viding mechanical support and maintaining the space for new tissue to develop. Key scaffold characteristics therefore include 3D structure with adequate porosity, biocompatibility, biome- chanical likeness, and biodegradability.[4] Both the chemical and physical properties of scaffolds are thus important in determin- ing their efficacy. Significant scaffold design criteria include material selection, biocompatibility, biodegradability, degra- dation profile (rate, by products, and strength characteristics), porosity (pore sizes, interconnections, and volume fraction), surface chemistry, topography, and cell–surface interactions.[5] As tissue and cell properties vary significantly around the body, the design of tissue engineering strategies including suitable scaffolds needs to be specific to the tissue type being targeted. The mechanical properties of bioengineered scaffolds vary sig- nificantly depending on architecture,[6] and studies have dem- onstrated that the interaction between cells and a scaffold can change depending on biomechanical properties.[7] Therefore, it is important to understand the mechanics of this tissue in its native state to design suitable scaffolds for engineering this tissue.

Both synthetic and natural biopolymers may be used for tissue engineering of soft tissues. The most commonly used polymers include polylactic acid, polyglycolic acid, and a combination of the two, poly(lactic-co-glycolic) acid (PLGA). Poly(lactic-co-glycolic) acid is a biodegradable synthetic poly- ester which is approved by the US Food and Drug Administration for human clinical use. Poly(lactic-co-glycolic) acid has been extensively investigated for uses in tissue engineering due to the versatility in fabrication and range of achievable chemical and mechanical properties.[8–10] Numerous strategies have been devel- oped to modify the surfaces of polymers like PLGA to improve their cell and tissue interactions and moderate the inflamma- tory reactions that occur when biomaterials are placed in the body.[11–13]

Natural polymers may also be used to create tissue engi- neering scaffolds and hydrogels. Commonly used natural poly- mers include chitosan, collagen, gelatin, silk fibrin, elastin, and glycosaminoglycans.[14–17] Such materials can be biocompatible,



Basic concept of tissue engineering involves the creation of a three-dimensional scaffold over which a precursor cell population is cultured.

provide favorable cell-binding sites and are often degraded through natural metabolic pathways in the body. Naturally derived polymers such as chitosan, possibly in combination with synthetic polymers for improved strength, have potential as tissue engineering scaffolds for soft tissues due to their bio-mimetic properties. Although known for their ease in forming macroporous structures, natural polymers such as chitosan can be limited in their mechanical stability. Cross-linking chito- san structures has been shown to improve stability of resultant scaffolds.[5]

More recently, foam-like material termed 3D graphene has been investigated as a potential scaffold material for tis- sue engineering.[18] Studies have since demonstrated high cell viability and enhanced oxygenation due to its highly porous structure.[19]

TISSUE ENGINEERING IN OPHTHALMOLOGY

Thus far there have been promising studies investigating to role of cellular and acellular bioengineered scaffolds to replace native tissue in corneal disease, conjunctival reconstruction, dry eye disease, and orbital fracture repair. These are discussed in more detail below.

Corneal Substitutes. Tissue engineering has long been investigated as an alternative to human corneal transplantation to treat potentially blinding corneal disease. There have been numerous studies of acellular polymer matrices aimed at promoting re-epithelialization in vivo. A number of groups have used Type I collagen scaffolds as artificial corneal extracellular matrices.[4,14] Griffith and

colleagues[20] have conducted a number of studies using fibrillar recombinant human collagen Type I and III (RHCI or RHCIII) as corneal stromal matrices. RHCIII was found to be optically superior, and the group was later successful in implanting 10 cell-free corneal substitutes into human patients made with RHCIII and cross-linked with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide and *N*-hydroxysuccinimide. After 6 to 7 months, the substitutes were well integrated with regeneration of corneal epithelium, stroma, and nerves, although long-term outcomes are unknown.[15,21] More recently, Zhang et al.[22] studied a novel collagen scaffold synthesized with rat tail

collagen I for use as a potential corneal tissue substitute for use in corneal transplantation. The scaffold was found to have comparable transmittance and thickness when compared with human cornea. Furthermore, the scaffold was successful in supporting reepithelialization and keratocyte cellularization ex vivo using porcine corneal epithelial cells.

Insler et al.[23] first reported the concept of corneal endo- thelial cell transplant expanded ex vivo onto collagen-coated dextran in 1990, and since then there have been many studies of corneal endothelial cell culture. Liang and colleagues[24] devel- oped a novel chitosan-based scaffold onto which they were suc- cessful in establishing corneal endothelial cell culture derived from rabbits ex vivo. Following implantation into Wistar rab- bits, the blended membranes demonstrated good histocompat- ibility and degraded steadily with less associated inflammation compared with control. Similarly, Ozcelik et al.[25] fabricated an ultrathin chitosan-poly(ethylene glycol) hydrogel film, which was found to be >95% optically transparent and able to support sheep corneal endothelial cell culture. Combinations of chito- san including keratin–chitosan and polycaprolactone–chitosan membranes have also been studied in vitro and been shown to support cell culture.[26,27]

Lai et al.[17,28,29] studied the use of corneal endothelial cell sheets fabricated with hydrogel carriers resembling the native corneal endothelium, which aimed to minimize some of the issues encountered with existing corneal endothelial substrates including optical interference, foreign body reaction, and dis- turbance of physiologic function. Initially working with gela- tin hydrogels, the authors recently investigated hydrogels made using hyaluronic acid, a biopolymer which is naturally found in the aqueous and vitreous. They found that cell sheet trans- plantation using these hydrogels resulted in superior biologic stability with minimal adverse effects in rabbit studies.[16] In an attempt to further improve the properties of collagen hydrogels, Takezawa et al.[30,31] developed a collagen vitrigel with the key step of vitrification allowing water to evaporate in a controlled manner with resultant cross- linking and rearrangement of col- lagen fibrils. The group later studied the ability of collagen vit- rigel to support the 3 main corneal cell layers, limbal explants, keratocytes, and endothelial cells, with promising results during in vitro experiments.[32]

Graphene has also been studied as a potential biomaterial for use in the cornea, with Tan et al.[33] culturing human corneal stromal fibroblasts onto graphene films for use as a synthetic keratoprosthesis skirt material.

Conjunctival Reconstruction. There have been a few studies investigating the use of tissue-engineered implants in conjunctival reconstruction. Hsu et al.[34] grafted porous collagen–glycosaminoglycan copolymer matrices into the bulbar conjunctiva of rabbits with artificial full-thickness conjunctival wounds. The authors found that by 28 days, the rabbits with matrix grafts had less wound contraction (6.8% ± 3.2% fornix shortening) compared with controls who were ungrafted (26.4%
± 5% fornix shortening). Lee et al.[35] later studied the use of modified PLGA 50/50 scaffolds modified with either hyaluronic acid or amniotic membrane in conjunctival reconstruction. The authors used human stromal fibroblasts obtained from human corneal tissues and were successful in seeding scaffolds prior to implantation in albino rabbits. At 4 weeks postoperative, grafted wounds were found to contract 6% compared with 25% of ungrafted conjunctival wounds. In addition to their use in the cornea, collagen vitrigels have also been studied in conjunctival reconstruction.[36] Zhou et al.[37] demonstrated that optimized vitrified collagen was able to successfully promote conjunctival epithelial cell growth and goblet cell repopulation during in vitro rabbit studies.

Dry Eye. There have also been a number of studies aimed at creating a tissue-engineered tear secretory device to treat patients with keratoconjunctivitis sicca who remain symptomatic despite conventional treatment. Many previous groups have reported successful animal and human cell culture onto basic extracellular matrices with collagen I and Matrigel (Corning Incorporated, NY, USA),[38–40] a preparation derived from basement membranes of the Engelberth-Holm-Swarm mouse sarcoma line containing laminin, collagen IV, heparin- sulfate proteoglycans, entactin, and nidogen.[41] Selvam and colleagues[42] have also since shown culture of purified rabbit lacrimal acinar cells onto numerous matrix protein-coated polymers including copolymers of PLGA (85:15 and 50:50) and poly-l-lactic acid with retention of secretory properties. These copolymers, as discussed previously, have the advantages of having adjustable biomechanical properties and the ability to be tailored to specific target tissues.

In a review of bioengineering for conjunctiva and dry eye, Lu et al.[36] discussed the potential use of “organ-on-a-chip” tech- nology for the ocular surface. Organ-on-a-chip refers to a bioen- gineered microdevice with cultured cells in an attempt to mimic target organ function, and some success has been reported with lung, liver, intestine, spleen, and bone marrow studies.[43–47] Any successful tear

~~secretory unit would require lacrimal gland cells, conjunctival epithelium, and microfluid channels~~, and prelimi- nary in vitro studies of conjunctival epithelium and artificial lacrimal glands provide a basis for further development.[48,49]

Orbital Fractures and Orbital Bone Engineering. The orbital floor is the most commonly affected wall of the orbit in trauma, and posttraumatic changes can manifest in enophthalmos and diplopia. Restoration of orbital volume is therefore vital in preventing complications and maintaining normal globe function. There are numerous implant options for use during orbital wall repair, of which autologous bone graft remains the gold standard, although with the obvious limitation of donor site morbidity and harvesting challenges. As such, various biomaterials have been

developed, and include nonresorbable alloplastic, resorbable alloplastic, and more recently, bioengineered bone. Nonresorbable material studied in the past includes titanium mesh, porous polyethylene (Medpor Stryker, Kalamazoo, MI, USA), and bioactive glass.[50] Notable risks of nonresorbable material include foreign body reaction, migration, and infection.[51] To address some of these issues, biodegradable polymers have been studied as alternative options. Poly(lactic acid), PLGA, and combinations of and derivatives of the two have been studied extensively in the past, and there have also been studies of polyglactin-910 mesh and a newer periosteum–polymer composite material.[50,52] Kontio et al.[53] compared polydioxanone and poly(l/d)lactide implants in rat studies and found that polydioxanone was mechanically unsuitable, losing form within 2 months but the poly(l/d)lactide polymers showed promising results at 7 months follow up. The group then progressed to human studies using poly(l/d)lactide 70/30 and found that the bioresorbable implants resulted in good clinical outcomes for patients with 2 cm^2 or larger defects with 36 weeks of follow up.[51] The authors then compared outcomes with fractures repaired using autologous bone graft and found no statistically significant differences in complications.[54] However, long-term outcomes of resorbable implants are not well described, and suitability of use may depend on fracture size.[50]

There have been a number of recent studies investigating the potential for orbital bone tissue engineering after previous established studies for bone regeneration of the mandible, cra- nium, and limbs.[55–57] Mesenchymal stem cells have been the most widely investigated cell line for craniofacial tissue engineering and have been shown to proliferate well in vitro from small sam- ples.[58] Additionally, there is a growing body of work surrounding bone morphogenetic proteins , which secrete signaling molecules stimulating differentiation of osteoprogenitor cells and thereby bone formation.[59] Currently, bone morphogenetic protein type 2and 7 have been developed for clinical applications.[60,61] Recent advances have used biodegradable 5-ethyl-5(hydroxymethyl)- b,b-dimethyl-1,3-dioxane-2-ethanol (EH)–poly(ethylene glycol) (EH–PEG) hydrogels with integration of mesenchymal stems cells to deliver bone morphogenetic protein-2 to injured tissue.[62] This is particularly of interest in orbital fracture repair, as the periosteum, which contains the osteoprogenitor and chondropro- genitor cells, is frequently injured in facial trauma, further delay- ing healing postfracture.[63] Betz et al.[62] loaded EH-PEG with bone morphogenetic protein-2 and implanted them into 8-mm orbital floor defects in rabbits. The authors found that there was significant bone growth at 28 days, establishing the viability of this concept for future studies. Rohner et al.[64] studied the use of polycaprolactone coated with bone marrow in pig orbital defects, which was shown to result in significantly more bone regenera- tion compared with polycaprolactone alone at 3 months postre- pair (14.1% vs. 4.5%). Medical grade polycaprolactone along with its composites created via fused deposition modeling has also been studied in orbital floor reconstruction with promising results in human patients.[65,66] Other

studies have focused more on craniofacial applications and include PLGA seeded with periosteal cells,[64] polycaprolactone with cultured calvarial osteo- blasts and mesenchymal progenitor cells,[67,68] and poly(propylene fumarate) scaffolds treated with growth factor and infused with bone marrow.[69] Such tissue-engineered bone constructs therefore have the potential to provide not only immediate support and restoration of orbital volume but also long-term benefits due to early stimulation of bone regeneration.

ROLE IN EYELID RECONSTRUCTION

Eyelid reconstruction represents one of the most challenging areas of reconstructive plastic surgery due to a combination

of anatomical complexity, functional considerations, and aes- thetic concerns. Eyelid defects requiring reconstruction are commonly secondary to tumor excision, trauma, or congeni- tal defects. Full-thickness eyelid defects that cannot be closed directly require reconstruction of both the anterior lamella, which consists of skin and the orbicularis oculi muscle, and the posterior lamella, which includes the tarsal plate and pal- pebral conjunctiva.[70] Among the most obvious structures to be bioengineered in eyelid is the tarsus, which is difficult to substitute and has excellent potential due to its thin structure. The tarsus provides both support and structural form, making it an essential component of the eyelid's function and physical appearance. Natural tarsus is a specialized tissue that features both dense fibrous connective tissue and typical cartilage. Structurally, tarsus consists of fibroblastic cells surrounded by an extracellular matrix with type I and III collagen, and aggrecan.[71]

An understanding of the target tissue biomechanics rep- resents an important first step toward successful tissue engi- neering. Important parameters to consider when evaluating biomechanics of structures such as the tarsal plate include the following: the elastic modulus, tensile strength, and maximum strain. The elastic modulus refers to the measured strain in being deformed elastically and is defined as the slope of its stress–strain curve. The tensile strength is defined as the maxi- mum stress or strain a material can withstand before failing. The maximum strain refers to the total strain just prior to fail- ure during tensile strength testing. In the only previous study of the biomechanics of human tarsus, we found that fresh tar- sus tissue had a mean toe modulus of 0.14 (0.10) MPa, elastic modulus of 1.73 (0.61) MPa, extensibility of 15.8% (2.1%), and phase angle of 6.4° (2.4°). After adjusting for the ini- tial tissue slack, the maximum strain ranged from 23.8% to 30.0%.[72]

There is only one previous study investigating the use of engineered polymeric scaffolds for tarsal repair, which was con- ducted prior to our study of the normal tarsus biomechanics.[73] This study used a type of polyhydroxyalkanoates as an acellular synthetic tarsal substitute in a rat study and found that they were successful in supporting eyelid reconstruction, fibroblast growth, and fibrous encapsulation. Polyhydroxyalkanoates are biode- gradable and thermoprocessable polyesters produced by micro-organisms.[39,74] Poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) consisting of 12% mol% 3HHx were used to produce scaffolds with an average thickness of 0.7 mm and resultant micropores of 5 μm diameter. These scaffolds were cut into 1 mm × 1 mm pieces and implanted into the upper eyelids of 3-month-old rats, with acellular dermal matrices of same size and thickness used as controls. Postoperative histologic studies demonstrated high density of inflammatory cell infiltrate around the poly(3- hydroxybutyrate-co-3-hydroxyhexanoate) scaffold in the first 2 weeks postoperatively compared with few inflammatory cells in the control group. At 8 weeks, the reaction had shifted to one of chronic inflammation, with ongoing macrophage and lym- phocyte infiltration with the

percentage of fibroblasts 32.13% ± 1.47% versus 100% in the acellular dermal matrix group and unoperated rates.[73] The significant inflammatory response dem- onstrated in this animal study suggests that further refinement is required to improve tolerability once implanted.

In addition to numerous studied strategies to improve the biocompatibility of engineered scaffolds,[11–13] cultured native cells preimplantation onto the scaffolds also aims to reduce such inflammatory responses. Given the importance of fibroblasts within the histologic structure of tarsus, seeding of these cells onto a bioengineered scaffold aims to both improve

biocompatibility and also enhance the biomechanical proper- ties. The culture of lung fibroblasts is well established in the literature,[75–78] and we have had success in replicating these studies with eyelid skin. Lung fibroblasts have been previously derived from lung tissue obtained at autopsy and cultured using Dulbecco's modified Eagle's medium (Life Technologies, Inc., Grand Island, NY) with various supplements at 37° in 10% carbon dioxide. Crystal violet staining along with immuno- fluorescent staining using a monoclonal antibody specific for human fibroblasts was used to confirm fibroblast culture.[79] Using a similar method, we have been successful in establishing fibroblast culture using small samples of eyelid skin taken at the time of various oculoplastic procedures. We found that cell culture reached confluence within 4 weeks and immunofluores- cent staining of the cells in early passages labeled strongly for fibroblast-specific markers. We are currently working on stud- ies aimed at establishing fibroblast cell seeding onto artificial scaffolds constructed with biomechanical properties similar to human tarsus tissue.

FUTURE CONSIDERATIONS

Bioengineering represents the future of reconstructive medi- cine, and there may be significant potential for the application in ophthalmic plastic reconstructive surgery.

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