Review

Tissue Engineering and Ophthalmology

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Abstract

Tissue engineering (TE) is a field of science that combines biological, engineering, and medical sciences and allows the development of disease models, drug development and gene therapy studies, and even cellular or tissue-based treatments developed by engineering methods. The eye is an organ that is easily accessible and amenable to engineering applications, paving the way for TE in ophthalmology. TE studies are being conducted on a wide range of topics, including the tear film, eyelids, cornea, optic nerve, glaucoma, and retinal diseases. With the rapid scientific advances in the field, it seems that TE is radically modifying the management of ocular disorders.

Keywords: Tissue engineering, gene therapy, disease model, drug development, regenerative medicine

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Introduction

Tissue engineering (TE) is a discipline based on the principles of biology, engineering, developmental biology, and the medical and morphogenesis sciences for tissue healing, treatment, and regeneration. TE aims to treat, repair, or replace damaged biological tissues and organs using cells and appropriate physiological factors combined with bioengineering, biomedical engineering, and materials sciences. TE also includes developing disease models, creating tissue scaffolds for cells, and administering active drug components to tissues.[1] Knowledge of how to control and regulate the intrinsic regeneration potential of the tissues is crucial.[2] The ultimate aim is to create artificial tissue or organ models to support medicine and the life sciences.

The eye tissues originate from epithelial, mesenchymal, connective, and neural tissue sources with precisely regulated structural and functional integration (Figure 1). TE has been used to achieve the above-mentioned objectives in the cornea, lacrimal gland, retina, optic nerve, and conditions like glaucoma, with significant progress from bench to bedside. The use of TE in ophthalmology includes, but is not limited to, the following:

a. Disease Models

Animal models may not faithfully recapitulate human pathogenic processes due to species differences.[3] With recent developmental milestones in microfluidic chips, stem cells, cellular signaling, and biomechanics, *organ-on-a-chip* technology has emerged from TE.[4] Microfluidic systems are designed to demonstrate the dynamic, functional, and pathophysiological properties of the tissues in living conditions. In a microfluidic system, specially designed channels are created in glass or polymeric materials, in which the biomaterials that constitute the tissue skeleton are seeded with specific cells that are cultivated to produce a three-dimensional (3D) structure that approximates physiologic conditions. These advanced engineered systems are

designed to recapitulate the unique microenvironment of organs *in vivo* and reproduce *in vitro* disease models.[4,5] Organ-on-a-chip systems can study the essential functions of one specific tissue/organ (i.e., "one-organ systems") or the interactions and reactions of multiple organs/tissues within a single system (i.e., "multi-organ platforms").

Recent advances have proven the feasibility of modeling human diseases with patient-specific induced pluripotent stem cells (iPSCs).[6] iPSCs are generated from a somatic cell line without using human embryos and can differentiate into any somatic cell. This represents a fundamental tool for studying disease pathogenesis and performing drug development studies (Figure 2).[7]

The organ-on-a-chip microfluidic technologies have increased our understanding of basic ocular physiology and disease pathogenesis. Overcoming the shortcomings of two-dimensional cell culture, they let us develop clinically relevant substitutes for eye treatment.[8] As an example, a microfluidic platform has been developed to dynamically cultivate the corneal epithelial barrier to study the effects of blinking shear stress on the human ocular surface and guide the development of ophthalmic drugs.[9] Similar systems can imitate and be used to evaluate the corneal epithelial wound repair process.[10] In another study, a microfluidic 3D microengineered cornea-on-a-chip was constructed based on primary mouse corneal epithelial and endothelial cells to realize basic corneal functions and facilitate research on topical drug delivery.[11]

In glaucoma research, TE approaches have been used to recreate the trabecular meshwork (TM) since the late 1980s. Cellulose porous filters with human TM cells grown on them, hydrogel molding, and photolithography techniques were used to study the effect of stiffness on gene expression and mechanotransducers, the mechanism of action of drugs on the TM, and drug repurposing.[12] Furthermore, a decellularized, tissue-engineered anterior segment eye culture was transplanted with TM cells to see if outflow structure and function would be restored. The engineered anterior segment scaffolds served as scalable ocular perfusion cultures to decrease the dependency on donor globes for outflow research and enable studies on perfusion cultures with specific genotypes and phenotypes.[13] A bioengineered synthetic 3D *in vitro* TM model can also be used to study detailed mechanisms of intraocular pressure (IOP) regulation to develop a glaucoma disease model and allow high-throughput screening of glaucoma drugs. This involves selecting and designing biomaterials for scaffold fabrication and extracellular matrix (ECM) components to mimic the trabecular architecture.[14]

Evaluation of retinal ganglion cells (RGCs) and their degeneration is also imperative in glaucoma research. TE methods to generate iPSCs from the blood or skin of glaucoma patients and induce them to differentiate into RGCs are valuable approaches in pathogenesis studies.[15]

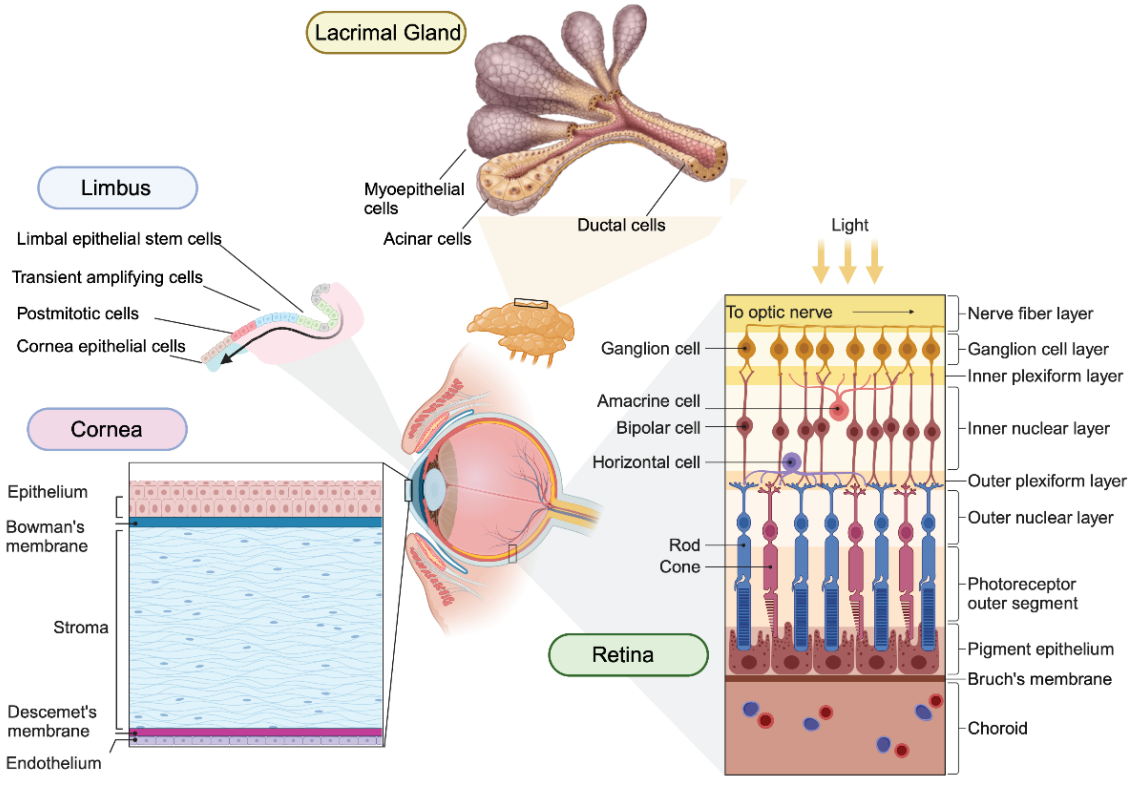

Figure 1. Schematic representation of ocular cell types and tissues. Diverse phenotypes and functions of ocular cells collectively establish and maintain vision

On the other hand, iPSCs can form self-assembled 3D structures that, upon differentiation, generate miniaturized organ mimicries named *organoids*. Organoids can recapitulate the cellular heterogeneity and architecture of organs and are excellent tools for studying the developmental phases of human tissues *in vitro*. Increasing interest in organoid research has also led to novel approaches in ophthalmology. iPSC-derived retinal organoids serve as 3D models of embryonic development, pathologies, and new therapeutic avenues for retinal diseases due to their high stability and similarity to native retinas.[16] Structures of the lens, neural retina, and retinal pigmented epithelium (RPE) cells have already been generated from undifferentiated embryonic stem cells (ESC) in a defined culture system.[17] Furthermore, 3D models of the human outer blood-retina barrier have been engineered to recapitulate critical features of healthy RPE-choriocapillaris interactions in dry and wet age-related macular degeneration (AMD) phenotypes.[18,19]

Optic nerve head (ONH) disease models have yet to be developed because of significant challenges which include the lack of a systems biology description of the ONH and large-scale gene expression and phenotype data in glaucoma, as well as insufficient knowledge about the roles of astrocytic and non-astrocytic ECM cells in tissue remodeling and the effect of actual IOP at the ONH on the tissues.[20] However, as iPSC-derived glaucomatous RGCs were shown to suffer from mitochondrial deficiency, improving mitochondrial biogenesis could be studied to reverse the disease process.[21] Merging more than one type of organoid results in more complex cellular structures named *assembloids*. Advanced *in vitro* models of diverse tissues resemble a more native-like environment, supporting and improving functional outcomes.

b. Drug Development Studies

TE provides a means for preliminary drug screening that reduces the need for highly criticized and expensive animal experiments, which are often poorly predictive of human physiology.[1] Indeed, in 2022 the FDA Modernization Act 2.0 eliminated the animal testing requirement for newly developed drugs before being given to humans. While the new law does not ban animal testing, it makes allowances for the use of new technologies such as artificial intelligence and organ-on-a-chip technology, where pharmaceuticals are tested on microchips that mimic organ function. Organ-on-a-chip models are expected to replace most drug and toxicity experiments in animal models.[22]

Bennet et al.[23] have grown immortalized human corneal epithelial cells in a microfluidic system to create a "corneal epithelial chip" to study eye drop transport. Another dynamic microengineered human corneal system has also been used for *in vitro* drug absorption studies.[24] A microfluidic plastic-based chip was used for *in vitro* 3D vascular network reconstruction in normal and pathological ocular angiogenesis studies and antiangiogenic drug testing.[25]

Drug repurposing is a feasible approach to identify new therapeutic uses for drugs approved for other diseases. The molecular mechanisms of action and the pharmacokinetic and pharmacodynamic properties of existing medicinal agents

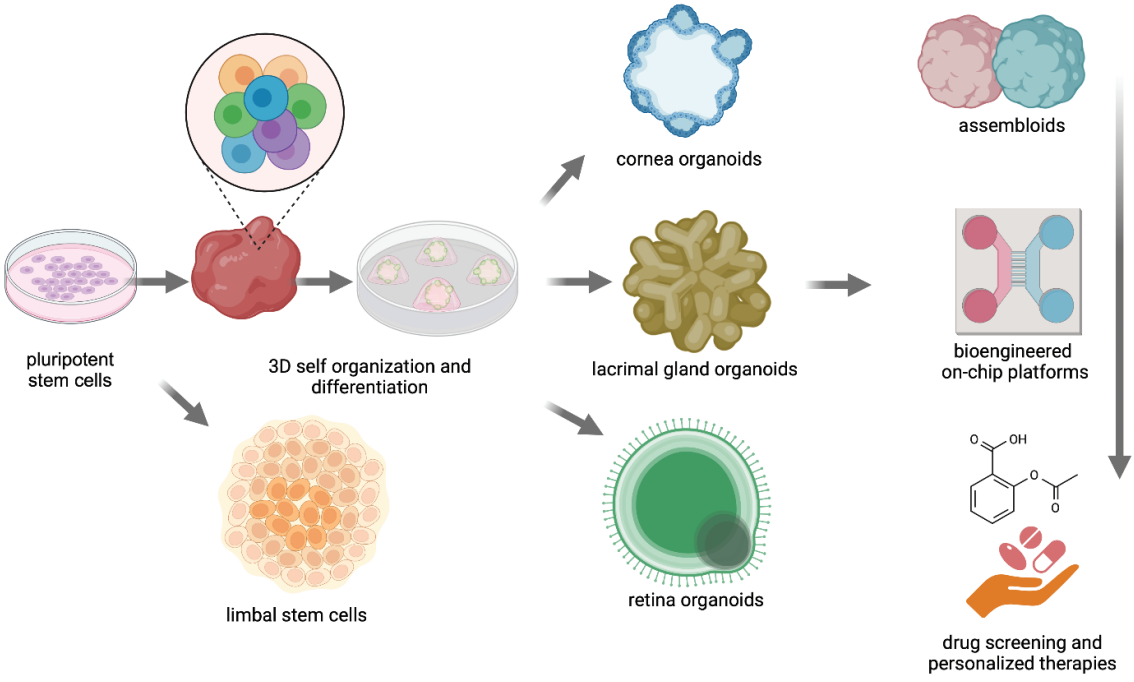

Figure 2. Pluripotent stem cells can give rise to all cell types, forming ocular cells and organoids. Ocular organoids and assembloids provide *in vitro* models to study the development of the eyes and related diseases. Bioengineered on-chip platforms can recapitulate physical and biochemical stimuli in a precisely controlled microenvironment, resulting in more realistic models in drug screening and personalized therapies in ophthalmology

are explored from the perspective of their pharmacological targets to redirect them to uncovered areas. The safety and efficacy of these drugs have already been established, saving time and cost. Structural modification, novel delivery system development, or combination with other medications are needed for some medicines requiring TE approaches. The development of controlled drug release systems is also possible.

The evolution of the use of cyclosporine A (CsA) in ophthalmology is an example of drug repurposing, per se. CsA was initially tested as an antifungal agent in the 1970s, with disappointing results. It was widely used in solid organ transplant surgery due to its potent immunosuppressive anti-T-cell potential and lack of bone marrow toxicity. Systemic CsA has also been used in noninfectious uveitis. Topically applied CsA was first used to inhibit experimental corneal allograft reactions in the 1980s. The beneficial effects of CsA in dry eye syndrome were initially noted in canine species.[26] Topical CsA has a local immunosuppressive effect in patients with Sjögren's syndrome, demonstrated by a significant reduction in CD4+ cells in both the conjunctival epithelium and substantia propria.[27] Today, it is one of the main treatment options for dry eye syndrome and various inflammatory lacrimal gland and ocular surface diseases.[28]

In glaucoma research, drug repositioning is among the most active fields.[15] The drugs being explored range from cholinergic medications to nifedipine due to vasodilatation of the ocular vascular smooth muscles.[29] In addition to IOP-lowering medications, the anti-epileptic drug valproic acid was repurposed as an adjuvant medication in glaucoma surgery. Valproic acid reduces collagen production and disrupts collagen fiber assembly in conjunctival wound healing postoperatively; thus, its anti-fibrotic activity might improve the success and prolong the survival of functional blebs.[30]

Retinitis pigmentosa (RP), AMD, and diabetic retinopathy could also be treated using repositioned medicines. In all neurodegenerative diseases, the shared signaling pathways leading to apoptotic cell death include calcium excitotoxicity, oxidative stress, mitochondrial dysfunction, and neuroinflammation. Targeting these main pathogenic mechanisms has successfully treated chronic diseases such as depression and epilepsies. The combinations include a list of possible repurposed drugs, such as brimonidine, curcumin, ceftriaxone, MitoQ, and valproic acid.[31]

Vision loss in dry AMD results mostly from RPE degeneration, partly driven by inflammation. Fluoxetine, approved by the FDA for treating clinical depression, inhibits the activation of inflammasomes and inflammatory cytokine release in RPE cells. Indeed, analyzing the data of more than 100 million Americans in two health insurance databases revealed a reduced risk of developing dry AMD among patients with clinical depression who had been treated with fluoxetine, suggesting fluoxetine as a potential drug-repurposing candidate for dry AMD.[32]

Another repurposed drug is dimethyl fumarate (DMF), which is approved for treating psoriasis and multiple sclerosis through its anti-inflammatory, immunomodulatory, and antioxidant effects. Vascular disorders of the eye, such as diabetic retinopathy and AMD, share a common pathogenesis associated with reduced nuclear factor (erythroid-derived 2)-like 2 (Nrf2) activity. DMF displayed induction of the Nrf2 pathway and related target genes, in addition to protection of photoreceptors, RGCs, and RPE. *In vivo* evidence has accumulated on its use in AMD, autoimmune uveoretinitis, cystoid macular edema, glaucoma, keratitis, optic neuritis, optic nerve crush, and retinal ischemia. Topical DMF administration needs to be studied for its repurposing in eye pathologies.[33]

Microfluidic systems have also been used to test surgical intraocular tamponades such as isopropanol silicone oil, which may emulsify and cause inflammation *in vivo*. An *in vitro* "eye-on-a-chip" microfluidic system showed that polymer silicone oil prevents decomposition and the formation of emulsion droplets.[34]

Topical treatments allow only a minority of the medicine to reach the ocular tissues. Developing *controlled-release ophthalmic drugs* aims to improve the effectiveness of drug delivery and reduce the number of times patients receive eye drops.[35] Biocompatible and biodegradable polymer particles capable of drug loading and controlled release enable non-invasive drug delivery. Poly(lactic-co-glycolic acid) (PLGA) is a frequently used biodegradable material.[36] For example, the anti-glaucomatous betaxolol or other beta-blockers combined with polymer ion exchange resin particles suspended in an adhesive medium is released more slowly and for a longer time in the eye (Japanese patent no: JPH0725698B2 - Formulation for the treatment of glaucoma with sustained release and comfortable application). Microfluidic chip studies might allow the preparation of polymer particles with uniform and controllable particle size, high drug loading, and suitable degradation. One such study involved the preparation of multidrug polymer particles by loading latanoprost and dexamethasone onto monodisperse biodegradable ~150-μm-diameter PLGA and examined the verification and optimization of drug release parameters.[37] Additionally, *drug nanosuspensions* with high bioavailability can be prepared. These are micron-sized drug particles suspended in a dispersed medium and stabilized by polymers or surfactants. For example, creating an ophthalmic hydrocortisone nanosuspension using a simple microfluidic nanoprecipitation method and nanocrystalization technology significantly prolonged its duration of effect.[38]

Combining microfluidic technology with current technologies can allow controlled *in vitro* drug delivery studies.[39] Precision *ophthalmic drug delivery devices* have mechanical, electronic, and microfluidic functions,[40] such as the contact lenses used as a platform for drug release to treat eye infections or release long-term steroids. It is crucial to adjust the composition of biomaterials to allow controlled drug release.[41]

The *in vitro* drug release studies in ophthalmology are mainly carried out under static conditions and do not consider the influence of flow dynamics of the tear volume. An *in vitro* 3D-printed eye model that included eyelids was developed to study fluconazole release from various commercial contact lenses.[42] It was designed to simulate physiological tear volume, as the volume of tears significantly speeds up drug release.[43] Another microfluidic unit was designed to simulate the tears' volumetric

flow rate.[44] The release kinetics of diclofenac were slower under dynamic conditions than static conditions. Furthermore, plasma-assisted grafting for surface modification of hydrophilic acrylic or silicon-based hydrogel materials with moxifloxacin significantly prolonged *in vitro* diclofenac release time to more than ten days.[45]

c. Gene Therapies

Gene therapies aim to alter cellular function via the delivery of genetic material, including DNA and RNA, or even proteins in some cases. Delivery can be performed *in vivo* or in cells that are removed from the body, transfected in *ex vivo* conditions, and returned to the organism later.

The cornea has the advantages of offering easy clinical access and an immune-privileged state for gene therapies, which focus on single and combination gene therapies via adeno-associated virus (AAV) and nanoparticle delivery. The potential applications of gene editing, such as Clustered Regularly Interspaced Short Palindromic Repeats/Associated Systems (CRISPR/Cas9), have been increasing.[46] Gene modulation therapies for endothelial diseases could make it possible to treat early-stage patients, reducing the need for corneal allografts.[47]

As for the posterior segment, modified gene therapy with AAV vectors was shown to be an effective neuroprotective and regenerative treatment that enhanced RGC survival and axon regeneration.[48] Voretigene neparvovec (Luxturna; Sparks Therapeutics) is based on a non-replicating AAV-2 vector that carries a modified transgene of human RPE65. It is the only approved gene therapy for treating dystrophies associated with biallelic pathological mutation of RPE65,[49] and the only approved for treating up to 1% of all RP patients.[50] The *RPE65* gene is responsible for visual cycle vitamin A metabolism, and its mutations cause approximately 6% of Leber's congenital amaurosis cases. The phase III clinical trial included patients with confirmed biallelic RPE65 mutations. Significant visual improvement with no serious adverse events and sustained improvement over 3-4 years of follow-up were reported after treatment.[51,52] Subretinal injection is the most frequent technique,[53] preferred over intravitreal injection because of its higher efficacy and lower systemic exposure.[54] However, the treated area is limited to the detached retina around the injection subretinal bleb.

d. Development of Biomaterials

One of the tasks in TE is to develop appropriate biomaterials with which the cells will interact. The biomaterials used in TE should meet certain criteria, including biocompatibility, structural stability, mechanical endurance, porosity to allow cellular integration, clinical applicability in the targeted tissue, and lack of toxicity, antigenicity, and mutagenicity.[55,56] In ophthalmology, transparency is also essential. Moreover, materials should not stimulate an inflammatory reaction and should potentially assist regeneration and healing.

Due to the cornea's complex structure featuring high refractive power and tensile strength with optical transparency, it is challenging to replicate its architecture using a single type of natural or synthetic biomaterial.[57,58] Corneal TE strategies have been primarily based on raw materials such collagen, gelatin, chitosan, amniotic membrane, and silk, as well as synthetic fabrics like polyvinylalcohol (PVA) and polyethylene glycol (PEG) derivatives. Natural biomaterials such as collagen have high biocompatibility, and gelatin has the advantage of being cheap, but their mechanical qualities are not up to standard. Decellularized cornea has similar properties to the native cornea but low bioactivity. Chitosan offers simple biofunctionalization, good biocompatibility, and manageable biodegradability. However, it must be crosslinked with other materials. As for synthetic biomaterials, the mechanical properties of PVA and PEG diacrylate (PEGDA) can be controlled, but both need additional components for corneal TE. Although collagen or amniotic membrane can be combined with PVA or PEGDA as a biocompatible biomaterial, together they might induce inflammatory responses. Combining chitosan with PVA or PEGDA yields proper mechanical properties but a lower-than-natural degradation rate.[59]

3D bioprinting is a technology that can be used to construct artificial target tissue scaffolds and imitate natural embryogenetic tissue formation with simultaneous processing of biomaterials and cells.[60,61] Using smart fiber alignment, keratocyte migration and orientation can be studied for corneal TE.[62] Conventional 3D printer heads are loaded with material into the cartridges as bioinks.[63] The extrusion-based method in a 3D printer seems the best to achieve enhanced mechanical properties. However, the droplet-based method may yield better microstructure, geometrical curvature, and enhanced elastic modulus.[63]

Nanotechnology in developing corneal scaffolds may promote cell adhesion, proliferation, and differentiation and facilitate gas, nutrient, and waste exchange in the corneal scaffold.[64,65] It is possible to enhance the functionality of the seeded stem cells, such as in chitosan nanoparticles/polycaprolactone membranes that yield a biodegradable scaffold for the maturation and growth of corneal endothelial cells (CECs).[66] Nanotechnology holds promise to personalize regenerative medicine in the damaged cornea.[67]

In addition to corneal TE, bioprinting technology was applied for the first time in 2020 to produce *tarsal plate scaffolds* using polycaprolactone, which was coated with adipose-derived mesenchymal stromal cells and seeded with sebocytes to secrete lipids, replacing meibocytes.[68] This technology might provide an alternative for treating massive eyelid defects, such as after tumor invasion or trauma.

The retina is the second most commonly studied eye tissue for bioprinting studies. The feasibility of creating 3D-printed scaffolds for retinal progenitor cells (RPCs) was demonstrated in 2017.[69] Chemically modified hyaluronic acid hydrogels and a Bruch's membrane-mimetic material have been synthesized.[70,71] The RGCs and glial cells successfully 3D printed on this scaffold remained viable with stable phenotypes.[72] When glial cells were used as a substrate for printed cells, the RGC neurite outgrowth was increased significantly.[73] Retinal disease models will be available once a complex retinal structure can be 3D-printed. As an example, an outer blood-retina-barrier 3D-printed tissue (i.e.,

RPE, Bruch's membrane, and choriocapillaris) has been created and is being studied to reveal the RPE-dependent choroidal phenotype in AMD.[74] A Müller cell-based 3D biomimetic model was also bioprinted and showed similar responses under hyperglycemic conditions as observed in an *in vivo* diabetic retinal model.[75]

e. Transplantation of Engineered Cells, Tissues, and Tissue Substitutes

Cell therapies as a subset of TE have been very promising in treating ocular diseases.[76] Stem cells are commonly used due to their plasticity and capacity to stay uncommitted and self-renewable until a signal to develop into distinct cell types is received.[77] ESCs are pluripotent stem cells that can renew through division and develop into the three primary germ cells. However, as ESCs can be potentially immunological and may be rejected, iPSCs are used as an alternative.

The success of cell therapy depends on using appropriate TE methods for cell processing and proper biophysical and biochemical stimuli to induce cells to migrate and settle in exact loci.[78] For transplanted cells to be functional, they must form new synaptic junctions and integrate with the host.[79] Certain cell types, such as human keratocytes, can be easily propagated using standard culture techniques.[80] However, the transfer and transplantation of cell lines may not be accessible in posterior segment applications. The eye is an electric-based organ; the retinal cells use endogenous electrical currents to function.[81] Electrical stimulation may improve the migration of transplanted cells, axonal regeneration, and synapse formation.[82]

Recent studies have focused more on developing artificial organs of clinically relevant sizes via TE methods using autologous or immunologically matched stem cells to address the growing need for biological tissue or substitutes.[1] With the current understanding of mechanisms in organogenesis and gene expressions, stem cell biology, and TE technologies, *regenerative medicine* has emerged. This involves isolating healthy cells from the organ in the initial stages of the disease, *in vitro* expansion of those cells, and seeding them on the tissue skeleton. Vital conditions for organogenesis are provided, and functional tissue is achieved in laboratory conditions. As the disease process resolves, the artificial organ is transplanted to replace the damaged organ. Despite the excitement about the possible use of ESCs and iPSCs in regenerative medicine, there are limited examples of actual translation into humans.

Combining a biosynthetic scaffold with cell culture-derived cells might allow a completely bioengineered cornea. Two main approaches have been used in corneal TE: to expand or create the desired cell population by tissue culture, or to support the corneal structure by providing a biomimetic/biosynthetic device that allows native cornea cells to stimulate endogenous corneal regeneration. In the first approach, the tissue culture of cornea cells can be transplanted as a sheet, or cells can be dissociated and injected into the desired part of the anterior segment.

These bioengineered corneas differ from the entirely artificial corneas (i.e., keratoprostheses) used in pathological eyes that cannot support a corneal graft. None of the keratoprostheses today integrates seamlessly into the host tissue[83] and none have addressed the reinnervation issue.[84] The quest for soft artificial corneas with improved biointegration, biofunctionality, and minimum complication rates continues.[85] Beyond the need for vision restoration, biointegration, epithelial overgrowth, and sensory innervation are crucial.[86] The strength and optical clarity of noncytotoxic, biosynthetic composites can be controlled. With further development, TE corneal replacements could address future donor cornea shortages.[87]

One artificial cornea product is worth mentioning. The CorNeat keratoprostheses has a synthetic polymeric scaffold for biointegration. It has a central poly(methyl methacrylate) optic and an integrating skirt, which imitates the microstructure of human ECM. Unlike scaffolds and other collagen matrices used in TE, it has mechanical strength and is nondegradable. The EverMatrix™ is optimized as a natural habitat for human fibroblasts, stimulates migration colonization, and synthesizes ECM. Initial animal studies confirmed biointegration[88] and human implantation revealed good medium-term results.[89]

Bone marrow mesenchymal stem cells (MSCs), adipose-derived adult MSCs, umbilical cord MSCs, and ESCs and iPSCs have been used for corneal stromal regeneration. These cells were implanted on the ocular surface, implanted intrastromally alone or with a biodegradable, non-biodegradable, or decellularized corneal stromal scaffold, injected into the anterior chamber, or injected intravenously. Stem cells from MSC banks may be autologous or heterologous. Even MSC exosomes were used for their immunosuppressive and damage-repairing effects to reduce the stromal scarring size.[90]

Rama et al.[91] were the first to try expanding adult limbal stem cells *in vitro* to treat limbal stem cell deficiencies. Stem cells in culture are identified as small cells that express stem cell markers such as ABCG2, are negative for cell differentiation markers like cytokeratin 3, and have a high nuclear to cytoplasm ratio. High expression of DNp63a is a quality control measure that allows successful transplantation.[91] Strict patient inclusion criteria with a milder host microenvironment are crucial for clinical success.[92] Cell therapies other than *ex vivo* cultivated limbal epithelial cells have been described,[93,94] including cultivated oral mucosal epithelial cells, extraocular MSCs, and iPSC-derived limbal stem cells.[95]

Expanded adult or stem cell-derived CECs have been studied to treat corneal endothelial diseases.[47] CECs are quiescent cells that must be pushed to proliferate. At the same time, it is necessary to avoid endothelial-to-mesenchymal transition, which might lead to a myofibroblastic phenotype and cellular loss of function. The alternative of differentiating CECs from iPSCs requires the development of strict protocols to ensure that the final output resembles CECs.[47] Strategies to deliver those cells or acellular endothelial graft equivalents are being studied to alleviate the need for allograft surgeries.[47]

Meanwhile, to repair the tissue of the lacrimal glands, the feasibility of epithelial cell adhesion molecule-positive progenitor cell injection therapy has been demonstrated.[96] Lacrimal gland stem cells cultured from the lacrimal glands of healthy and

aqueous deficiency dry eye disease mice express progenitor cell markers (i.e., Krt14, Krt5, P63, nestin),[97] have self-renewal capacity, and differentiate into acinar or ductal-like cells *in vitro* and *in vivo*.[98] Conversely, a 3D TE technique with a direct reprogramming method to induce markers in the developmental process from human iPSCs has regenerated a secretory gland structure by reproducing embryogenesis *in vitro* and *in vivo*.[99] *In situ* regeneration of the partially damaged lacrimal gland is possible using stem cells in the glandular tissue. Organoids developed from iPSCs with TE methods can be transplanted for total glandular damage.[100] Indeed, the generation of organoids from iPSCs committed to neuro-ectodermal lineage displayed acinar, ductal, and myoepithelial structures specific for lacrimal glands and confirmed secretory function.[101]

For retinal stem cell therapy, retinal organoids seem to be a helpful resource.[77,102] RPCs are multipotent stem cells with mitotic capability, found in the neural retina of human fetuses between 16 and 20 weeks of gestation.[103] If manipulated *in vitro*, they can express photoreceptor markers, differentiate into neuronal cells of the retina,[104] and integrate into the outer nuclear layer of both intact and degenerating retinas in adult mice.[105] Timely selection of biochemically committed but not yet morphologically differentiated progenitors gives the best results.[106] RPCs at the peak of rod genesis can differentiate into rod photoreceptors when transplanted and form synaptic connections to integrate into the degenerating retina.[106,107] RPCs are transplanted subretinally or intravitreally. However, as in MSC procedures, subretinal implantation techniques are not risk-free.[108] Clinically, Stargardt's macular dystrophy, dry AMD, RP, and possibly retinal vascular diseases could benefit from ESCs.[109,110]

Replacing damaged RPE with healthy iPSCs can delay disease progression.[111,112] Transplantation of allogeneic or autologous RPE cells or sheets has been studied.[113] In a swine model, iPSC-derived RPE cells injected into the atrophic retina retained their typical morphology, RPE-related gene expression, and phagocytic ability.[114] Combined RPE and retinal sheet transplantation shows potential for complete replacement of the degenerated retina.[115]

Other stem cells have also been studied for RP with beneficial results, including the use of human iPSC-derived retinal cells in mice, bone marrow stem cells, and even conjunctival MSCs.[50,116,117,118] *In vivo* animal studies demonstrated that transplanting MSCs into the vitreous can improve photoreceptor survival in RP.[119]

Glaucoma and other optic neuropathies are the most common diseases expected to benefit from RGC replacement therapy.[110] However, donor neuron survival and retinal integration issues must be considered. Human iPSCs could be differentiated into RGCs, and aligned nanofiber matrices could be used for *in vitro* optic nerve-like modeling to guide the axonal outgrowth of these iPSC-derived RGCs. Forskolin, a cholinergic derivative, is also known to promote RGC differentiation.[120] Protocols for *ex vivo* human RGC transplantation for research on donor survival, dendritic stratification, topographic distribution, and donor-host interactions have been reported and hold promise for future applications.[121,122,123] When human iPSCs were differentiated into mature, functional RGCs *in vitro* and transplanted intravitreally in mice, their retinal localization, morphology, and functionality were similar to native RGCs.[124] A porcine decellularized optic nerve seeded with neurotrophin-3-overexpressing Schwann cells was shown to serve as a functional scaffold and promote directional growth and remyelination of regenerating axons, thus proving successful as an *in vivo* spinal cord defect model.[125]

On the other hand, Wharton's jelly-derived MSCs (WJ-MSCs) have been studied in terms of the effects of secretory exosomes, not neuronal transformation.[126] They are known to increase mitochondrial adenosine triphosphate synthesis and suppress neuroinflammation.[127,128] Umbilical cord WJ-MSC therapy has been suggested for the management of toxic optic neuropathies if performed soon after the incident and for RP, regardless of the causative mutations.[129,130]

3D Microtissues

3D culture systems ultimately aim to enhance cell-cell interactions and facilitate native-like microenvironments, thereby emulating tissue physiology. Cellular spheroids or 3D microarchitectures can either be formed naturally by aggregation of cells in hanging droplets or templated molds due to gravitational forces or by utilizing bioengineered platforms such as photolithography, droplet-microfluidics, or bioprinting.[131,132,133] Injectable ophthalmic microtissues have been generated from conjunctival stem cells to treat ocular surface diseases.[131] The 3D dynamic culture enabled constructs to maintain their stemness and facilitate efficient ocular differentiation. Hirayama et al.[134] successfully demonstrated the bioengineering of a functional mouse lacrimal gland from a microgerm composed of epithelial and mesenchymal cells. Following orthotopic implantation, the generated lacrimal germ could secrete tear proteins such as lactoferrin upon stimulation with pilocarpine. Similarly, spheroids generated in patterned molds and composed of lipoaspirate-derived pluripotent stem cells and MSCs were capable of preserving their differentiation capacity and restoring the cornea stroma in animal model.[132]

Regulation, Commercialization, and Ethics

The regulations on using and commercializing bioengineered products in humans are still being developed as new products emerge. However, using ESCs and animal experiments to create new therapeutic agents has been subject to severe ethical criticism for years. Using organ-on-a-chip technology for disease models might decrease the need for animal experiments by drugmakers. Ethical concerns in using human embryos and problems in matching patients based on compatible blood types can be overcome by using iPSCs. However, there are still safety concerns related to epigenetic memory, where derived cells retain gene expression from the original cells, and the ability of iPSCs to proliferate indefinitely, which can possibly lead to teratomas.[50]

Ethics

Authorship Contributions

Concept: C.A.U., S.G., Design: C.A.U., Data Collection or Processing: C.A.U., S.G., Literature Search: C.A.U., S.G., Writing: C.A.U., S.G.

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