Required Properties of the Corneal Endothelial Implants

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Treating corneal diseases arising from injury to the corneal endothelium necessitates donor tissue, but these corneas are extremely scarce. As a result, researchers are dedicating significant efforts to exploring alternative approaches that do not rely on donor tissues. Among these, creating a tissue-engineered scaffold on which corneal endothelial cells can be transplanted holds particular fascination. Numerous functional materials, encompassing natural, semi-synthetic, and synthetic polymers, have already been studied in this regard.

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1. Introduction

Incoming light is transmitted and focused onto the retina via the cornea, which is located at the front of the eye and has no blood vessels and good transparency. A cornea consists of five distinct layers, with the corneal endothelium (CE) positioned as the posterior layer. The CE plays a crucial role in maintaining corneal transparency by regulating the dynamic hydration of the cornea, which is achieved through a delicate balance between a “leaky” barrier and active ionic pumps located on the corneal endothelial cells (CECs). Injury or dysfunction of these cells may result in impaired ion and solute transport, thereby causing corneal edema. The primary difference between the corneal endothelium and other bodily tissues or cells is the fact that CECs lack the ability to regenerate in vivo [1]. Therefore, it is crucial to maintain their health throughout life to prevent corneal edema.

Over 10 million individuals around the world are said to be affected by corneal disorders, which are the fifth most common cause of blindness. Currently, corneal transplantation is thought to be the best course of treatment for these conditions and is one of the most commonly performed transplant procedures worldwide. While endothelial dysfunction is the primary reason for corneal transplantation, corneal dystrophies are the primary cause of this dysfunction. Fuchs’ endothelial corneal dystrophy is the most prevalent corneal dystrophy, which contributes to around 39% of all corneal transplantations performed globally [2].

Penetrating keratoplasty and endothelial keratoplasty (EK) are the two primary kinds of corneal transplantation. Penetrating keratoplasty entails replacing all layers of the recipient’s cornea with a donor cornea, while EK selectively replaces only the defective posterior layer of the cornea [3]. According to the 2021 Eye Bank Association of America report, more than sixty percent of all corneal transplants were EKs, making it the most popular keratoplasty [2].

One major predicament pertaining to keratoplasty is the restricted accessibility of appropriate donor tissue. It has been statistically established that the quantity of patients necessitating medical intervention is significantly greater than that of available donors [2]. Additionally, an obstacle associated with cadaveric donor transplantation is the prolonged risk of rejection and malfunction of an allogenic graft [4]. Considering that the endothelium is the most impaired and delicate tissue, it is imperative to explore additional possibilities, such as tissue engineering methods in vitro for corneal endothelium regeneration. Current options comprise cell therapy or combining cells with artificial substrates. Researchers can extract CECs from donor corneas by enzymatic digestion and achieve in vitro expansion of CECs using a medium that promotes cell expansion in vitro (e.g., medium containing specific growth factors) to activate signaling pathways (e.g., phosphatidylinositol 3-kinase (PI3K)/Akt and Smad2 signaling pathways), resulting in the proliferation of CECs [5]. Once these CECs are generated, they must either aggregate with one another to create a transplantable cell sheet or be combined with extracellular matrix (ECM) components, allowing later implantation. In the latter scenario, these cells must directly adhere to a substrate akin to Descemet's membrane (DM). Biomaterials that can enhance cell function may be developed to improve the durability of transplanted grafts and the proliferation of CECs in vitro.

2. Required Properties of the Corneal Endothelial Implants

Descemet's membrane (DM) is an extracellular matrix situated posteriorly to the cornea and secreted by the corneal endothelial cells. Its main components include laminin, fibronectin, type IV and type VIII collagens, and perlecan. DM functions as a basal lamina for the corneal endothelial cells in a human cornea. To enhance the in vitro proliferation of corneal endothelial cells, promote the successful implantation of the scaffold, and optimize its endothelial functional capacity, it is imperative to ensure that the implants exhibit performance characteristics as similar as possible to those of DM. The basic properties include transparency, permeability, mechanical stiffness, topography, biocompatibility, degradability, etc.

The primary characteristic is transparency, which pertains to the corneal endothelium's capacity to transmit light. DM exhibits a high level of transparency, reaching up to 90% within the visual range. The natural cornea's refractive index is 1.367, which is critical to the refractive power of the eye. Refractive errors could occur even if its contour is slightly changed [6]. The implant must possess a refractive index that is as similar as possible to the corneal tissue and an adequate endothelial cell density to preserve the cornea's normal light transmission (≥90%).

Subsequently, permeability should be taken into account. Permeability pertains to the capability of allowing nutritional elements to flow through the CECs. Aqueous humor provides most of the nutrients the cornea and the water need to preserve their typical hydrated states. Despite the fact that the cornea lacks blood vessels, the endothelium establishes a route for water and nutrients to receive from the anterior chamber to the cornea. Consequently, corneal endothelial implants must be permeable to biomolecules providing nutrients such as glucose and albumin. Generally, molecules with smaller molar masses exhibit greater permeability than those with larger molar masses. Glucose is a low-molecular-weight compound (0.18 kDa) and is considered the primary source of energy transfer in the cornea. It is present in the aqueous humor, penetrates through the endothelium into the corneal stroma, and metabolizes to produce lactic acid that subsequently diffuses back into the anterior

chamber. The scaffold materials' permeability can be evaluated by using the diffusion coefficient of glucose through the graft. As there is limited data in the literature on the permeability of DM, the corneal diffusion coefficient of glucose can be used as a reference value ($3.0 \pm 0.2 \times 10^{-6}$ cm^2 s^{-1}).

Mechanical properties are indeed essential for corneal endothelial implants. Specifically, mechanical stiffness refers to the ability of an implant to maintain its shape and resist deformation. This property can be expressed in terms of tensile strength, Young's modulus, elongation properties, and other similar measures. Since an implant serves as the support for the CECs, it must be able to withstand mechanical stretching and folding during implantation through small incisions in the eye while still retaining its shape and mechanical, physical, and optical properties once it is unfolded in situ. In addition, the implant should possess adequate viscoelasticity, which enables it to adapt to the changing curvature of the cornea during the healing process from its edematous state to the post-edematous state. The stiffness of an implant can significantly impact the behavior of the cells that adhere to it. Therefore, the mechanical strength of an implant should be similar to that of DM in order to provide an environment conducive to the expansion of natural corneal endothelial cells. Previous research has indicated that DM exhibits a tensile strength of ~2.6 MPa and Young's modulus of ~50 KPa [7]. The thickness of the scaffold implanted by DMEK is 10–20 µm [8]. It is a daunting challenge to maintain mechanical resilience with such thickness.

The substrate of an implant should ideally provide a suitable microenvironment that can support the viability, proliferation, and signaling pathways of CECs. To achieve this, the matrix used for culturing CECs should replicate the molecular, physiological, and mechanical properties of DM. This promotes cell expansion, cell adhesion, the deposition of the extracellular matrix, and the interactions between the cell layer and the scaffold. Topography is a key physical factor that can guide stem cells' fate by providing surfaces with specific roughness that can influence the behavior of the attached cells [9]. The necessity of subtle surface roughness to enhance cellular behavior is attributed to the amplification of protein adsorption onto the substrate's surface, which inherently boosts the CECs' interaction with the substrate [10]. Various morphologies are fabricated through techniques such as replica molding or stereolithography [11]. In recent work, 3D confocal microscopy was employed to analyze the microtopography of DM [12]. The study revealed irregularly shaped, flat hexagonal pits that gave CECs a distinctive polygonal or hexagonal appearance. The pits had a width ranging from 10 to 20 µm and were up to 1 µm deep. According to the findings, CEC functionality was improved by replicating the native DM structure. Earlier research found that the growth of CECs varied with topographic features. When the topographic height and density of the substrate surface were low, CECs tended to form continuous monolayers more often. Still, this ability was diminished on substrates that were tightly packed. As a result, topographical cues might be employed to forecast CECs' capacity to construct the required continuous monolayer [13]. Furthermore, achieving an optimal balance between hydrophilicity and hydrophobicity is crucial for promoting cell-surface interactions. Surface wettability and the properties of functional groups, such as carboxylic acid and amine groups, are also crucial factors that significantly influence cell adherence and modify the microenvironment for CECs [14].

Corneal endothelial implants should maintain continuous attachment to the posterior surface of the cornea. Research suggests that the hydrophilic external surface of the implant can support adherence to the cornea,

eliminating the need for sutures or an additional binding agent [15]. During the healing process, the long-term adherence of an implant to corneal tissue is facilitated by bio-active attachment through protein adsorption, primarily fibronectin and laminin. Furthermore, the clever design of an implant shape, including the center and skirt, can also enhance its adhesion to the cornea.

The hydrophilic/hydrophobic nature of the materials is also important for corneal endothelial tissue engineering scaffolds. Hydrophilic materials commonly include gelatin, chitosan, polyethylene glycol (PEG), collagen, etc. [14][16][17]. Apart from promoting the adhesion of transplants to the posterior cornea, hydrophilic materials can help with the penetration of nutrients such as glucose and the adsorption of proteins. They have a significant role in promoting cell adhesion because of their high water absorption capacity. Hu et al. [17] reported the fabrication of gelatin/polycaprolactone (PCL) and collagen/PCL nanofiber scaffolds, demonstrating expected hydrophilicity, wettability, and biocompatibility. Bone marrow endothelial progenitor cells (BEPC) adhered well to collagen/PCL and gelatin/PCL scaffolds. On the other hand, hydrophobic materials used for corneal endothelial scaffolds include polycaprolactone (PCL), poly(D,L-lactic acid) (PDLLA), etc. [18][19]. Due to their hydrophobicity, nanofibrous membranes made from PDLLA have relatively low glucose permeability [18]. Additionally, the hydrophobicity of PCL makes it have a slow degradation rate, which plays a crucial role in modulating the degradation properties and mechanical properties of chitosan nanoparticle/PCL composites [19].

Both biodegradable and non-biodegradable materials can be utilized for corneal endothelial grafts. If the graft is designed to be permanently present in the body, then a non-degradable scaffold material, such as polymethylmethacrylate (PMMA), can be employed. Kruse et al. [18] reported using an electro-spun PMMA scaffold as a carrier for CECs, which exhibited cytotoxicity. The long-term safety and inflammatory response of non-degradable scaffolds in vivo may be of concern. As the CECs continue to secrete an extracellular matrix, the thickness of the DM increases over time, which is expected to replace the scaffold. Therefore, biodegradable materials are often studied as part of corneal endothelial tissue engineering scaffolds. If a material is biodegradable, its degradation rate must be synchronized with the rate of DM regeneration to avoid any adverse effects on the rest of the eye. Controlled biodegradation of the tissue-engineered matrix without producing toxic byproducts is crucial to restoring the natural structure, morphology, and function of the target tissue. The implanted materials and their breakdown products must be easily reproducible and have non-toxic qualities. Most natural materials, such as gelatin and chitosan, are degradable in vivo. At the same time, the most commonly used synthetic material is polycaprolactone, which has a relatively slow degradation rate. Tayebi et al. [19] constructed a biodegradable transparent scaffold for culturing corneal endothelial cells by incorporating chitosan nanoparticles into chitosan/polycaprolactone (PCL) membranes. The in vitro degradation of the chitosan nanoparticles/chitosan/PCL composite scaffold was tested by immersing the scaffold fragments in PBS (pH = 7.4) at 37 °C. The slope of the degradation rate of the scaffold was approximately constant over 21 days, and ultimately, approximately 76% of the scaffold degraded. The shape of the scaffold was almost preserved throughout the degradation assessment. Song et al. [20] combined poly(lactide-co-caprolactone) with an extracellular matrix to construct a transparent, biodegradable adhesion carrier for CECs. It is known that the long-term biodegradation

profile in vivo is a key issue for transplanting biodegradable polymers or scaffolds in vivo. Unfortunately, it is difficult to precisely track the rate of in vivo biodegradation of synthetic polymers.

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