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Approaches to corneal tissue engineering: top-down or bottom-up?

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Abstract

Tissue engineering creates biological tissues that aim to improve the function of diseased or damaged tissues such as the cornea (the main refractive component of the eye). Traditional tissue engineering strategies employ a ''top-down'' approach, in which cells are seeded on a polymeric scaffold that they then populate and create the appropriate extracellular matrix (ECM) often with the aid of perfusion, growth factors and/or mechanical stimulation. However, in highly organised tissues, such as the cornea, top-down approaches have difficulty recreating intricate but necessary microstructural features.

With the desire to create more complex corneal tissues with features such as anisotropic hierarchical molecular assemblies, appropriate mechanical properties, cell binding motifs and corneal specific morphology, we are developing tissue engineering techniques that are moving away from the traditional top-down approach and instead focusing on building modular micro-tissues with repeated functional units which facilitate a bottom-up approach.

Here we report on the success and shortcomings of both top-down and bottom-up approaches to creating engineered corneal tissues. Specifically, we will discuss recent work demonstrating the importance of engineering corneal ECM with appropriate levels of tissue compliance using a top-down approach. We will then highlight a bottom-up approach, which focuses on fabricating discreet bio-prosthetic ECM building blocks (corneal lamellae) with specific micro-architectural features derived solely from human corneal keratocytes under serum free conditions using enzyme responsive templates. These building blocks will then be used to generate a whole cornea whilst maintaining the intricate architecture and complexity of native corneal ECM.

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1. Introduction

The cornea is the main refractive part of the eye responsible for over 75% of light transmission to the retina thus enabling vision [1]. The cornea is transparent and comprised of three cellular layers: an outermost epithelium, a

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middle stroma and an innermost endothelium [2-3]. Although highly innervated, the cornea is avascular and immunologically privileged.

According to the World Health Organization, "diseases affecting the cornea are a major cause of blindness worldwide, second only to cataract in overall importance" [4] and allograft cornea transplantation remains the most common treatment. However, the supply of high quality donor cornea worldwide falls well short of demand, moreover they present a low but genuine risk of disease transmission, largely circumvented by expensive screening procedures ($2,500 - $3,500 USD per cornea) [5]. These complications are compounded by the growing use of corrective eye surgery, which renders these corneas unsuitable for grafting, further reducing the availability of acceptable allogeneic supplies [6]. As such the replacement of allograft tissue has become a growing topic of interest, and there have been many versions of artificial replacements loosely grouped under "artificial corneas" that have been developed. These range from prostheses, known as keratoprostheses (KPro) to regenerative medicine approaches which typically combine cells with transparent materials but also include decellularized corneas.

The concept of the artificial corneal substitute was introduced in 1789 by Guillaume Pellier de Quengsy as a keratoprosthesis (Kpro) with a porous prosthetic skirt [7]. This design proved to be fundamental to artificial cornea research and is currently the model used for the Chirila Kpro, AlphaCor^{TM} [8-11], one of the most clinically successful corneal substitutes. The biocompatibility of the microporous skirt material is key to the success of Kpro in repairing the cornea. Such microporous materials include poly(2-hydroxyethyl methacrylate) [11,12], porous nano-hydroxyapatite/poly [13-16] and collagen hydrogels [17-29] each ensuring improved biocompatibility of Kpro. Collagen hydrogels, in particular, have replaced less biocompatible materials used for the construction of biocompatible Kpro skirt.

Collagen type I, the most abundant stromal protein in the cornea [30], it is biocompatible, biodegradable and possesses low immunogenicity. Therefore hydrogels of collagen type I are particularly attractive as matrix replacement scaffolds, partly because of their strength at relatively low concentrations, resulting from the virtually rigid rod properties of the collagen type I triple helix [31]. In addition, collagen contains the cell attachment motif arginine-glycine-glutamic acid [32]. Collagen hydrogels are, however, unstable and hence require stabilization, e.g. by chemical crosslinking [25] or plastic compression [33,34]. Despite the problems associated with collagen gels our thesis remains that it is the most appropriate material for corneal tissue engineering.

Armed with the belief that collagen remains the most suitable material from which corneas can be engineered we have explored a number of manufacturing approaches that have been grouped into top-down and bottom-up. In the bottom-up approach there are multiple methods for creating modular tissues, which are then assembled into engineered tissues with specific microarchitectural features. In the top-down approach, cells and biomaterial scaffolds are combined and cultured until the cells fill the support structure to create an engineered tissue. Both approaches to corneal tissue engineering are discussed below.

2. Top-down approach to corneal tissue engineering

Previously we have explored a practical method for enhancing the mechanical strength of collagen type-I gels via plastic compression, which eliminates the majority of the water content from typical, relatively hydrated, collagen gels. By varying collagen concentration and compression times, we constructed a stronger collagen-based substrate. Specifically, we performed controlled, unconfined plastic compression to rapidly produce dense, pliable, mechanically-strong collagen scaffolds with limited but controllable micro-scale features [26]. In order to mimic the normal corneal stroma corneal keratocytes were embedded in the collagen scaffold before compression and once compressed preserved the cells in a viable cornea-like form [24,26] suitable for corneal transplantation [35]. Transparency was shown to improve within the construct when embedded cells were grown in serum-free media (Fig. 1). Moreover, this engineered structure provided an improved substrate capable of

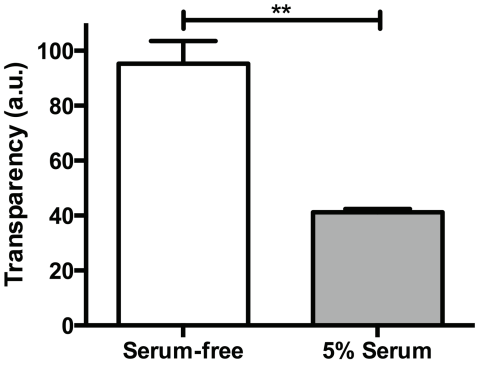

Figure 1. The transparency of tissue engineered constructs developed from compressed collagen gels containing human keratocytes can be improved by cultivation in serum-free media.

supporting the expansion and stratification of corneal epithelial cells. Whilst this top-down approach continues to gain much clinical and commercial attention we have subsequently focused more on its application as an in vitro model of the corneal stroma. Indeed we have had great success in using this approach to create artificial corneas with tractable levels of compliance (tissue stiffness) and subsequently shown that corneal epithelial stem cells are sensitive to the compliance of the substrate upon which the reside. Similar to studies using mesenchymal stem cells [36] we have shown that that a more compliant substrate maintains the corneal epithelial cells in an undifferentiated form and that if subsequently grown on a stiffer (less compliant) surface they readily differentiate [37-39]. The biological consequences of this work are only starting to be fully realized. Based on this work we have recently put forward the hypothesis that there is a gradient of increasing stiffness centripetally across the cornea and it is this change in compliance that drives both the direction of migration as well the differentiation of corneal epithelial cells during homeostasis [40]. Moreover the application of this phenomenon is the focus of ongoing regenerative medicine research in our lab.

3. Bottom-up approach to corneal tissue engineering

Directing cell behaviour on two-dimensional surfaces so that they perform as if they were in a natural three-dimensional tissue represents a significant challenge, but one that must be met if the early promise of cell and tissue therapy is to be fully realised. The scaling up of cell-based therapies needs to be based on two-dimensional culture systems if it is to be commercially viable but it is well understood that the three-dimensional structure of cornea is essential for its proper function. While cells on rigid, planar surfaces can respond to the chemical and mechanical nature of the *ex vivo* culture system, they typically have little capacity to reproduce a tissue-specific extracellular matrix (ECM). Furthermore, and unlike cultures in three-dimensional matrices (i.e. using top-down approaches), cells on flat, non-functionalized surfaces do not exhibit native phenotypes, which consequently affect their ability to form higher order structures [41,42]. Therefore, a biomaterial through which one can translate sophisticated two-dimensional (2D) experimental design into complex cell-derived 3D (hierarchical) structures is of great value to a modular, bottom-up approach to corneal tissue engineering. To this end we have recently used RGD(S) peptide amphiphiles (PA's) to coat hydrophobic surfaces composed of striated polytetrafluoroethylene (PTFE) to obtain a highly organized tissue, with strict orientation of human cornea stromal fibroblasts (hCSFs) and corresponding deposits of extracellular matrix similar to that observed three-dimensionally *in vivo* [43]. These PA's have been synthesized as self-assembling molecules to obtain supramolecular structures with geometrically-defined nanoscale patterns at distinct surface densities, which can be significantly higher than those achieved with intact matrix macromolecules [44,45]. The use of supramolecular self-assembly offers the possibility of controlling the structure, topography, shape, and dimensions of the biomaterial, as well as the spatial display and density of the bioactive motifs. This is made possible by the local order in the assembled nanostructures [44,46]. Previously, several different forms of PA nanostructures have been used by us to direct stromal cell growth [43, 47-54]. Of particular relevance to the bottom-up approach is the use of enzyme sensitive RGD-PA [54]. These PA's also form a coating upon which human corneal stromal cells (keratocytes) can attach, align and form organised collagen structures. However they have the additional capability of responding to specific endogenous proteases. Once the cells have been directed to form an organised collagenous corneal matrix of up to 10μm thick, matrix metalloproteinase expression is upregulated by removal of retinoic acid from the culture media

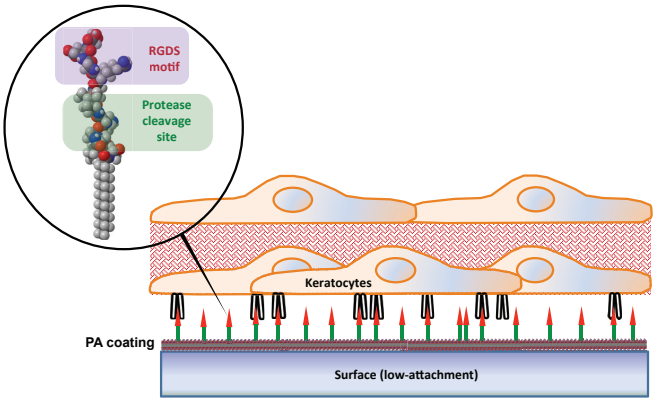

Figure 2. Human keratocytes grown in serum free conditions upon a dual functional bioactive peptide amphiphile allows for the controlled attachment, stratification and eventual enzyme driven release of an engineered tissue. Protease expression is controlled by retinoic acid supplementation to the media.

[55]. This increased protease expression cleaves the PA between the hydrophilic cell-binding head group and the hydrophobic tail of the enzyme-responsive PA resulting in the newly formed piece of engineered cornea to become free of the 2D surface. These lamellae like constructs are then ready to be stacked to form a corneal tissue with the required level of thickness (Fig. 2).

4. Conclusions

There have been significant developments in tissue engineering approaches to replace partial or full thickness damaged or diseased corneas. Biomaterials have been developed to assist in these reparative procedures, to restore minimal function or to regenerate the cornea to different degrees. We posit that collagen, as the main structural component of the cornea, should remain the biomaterial of choice for corneal tissue engineering. We have demonstrated that collagen can be used as a direct scaffold on to which cells are seeded (top-down) or it can be endogenously produced via application of interactive biomaterials to control the spatiotemporal cell position and differentiation (bottom-up). Whilst both approaches have merit the heightened degree of sophistication in both the design of the biomaterials used (e.g. enzyme-responsive peptide amphiphiles) and resulting human derived tissue equivalent places the bottom-up approach as our preferred choice. Time will tell as to which approach will have the greater clinical success, this is likely to be guided more by limitations in bioprocessing and cost-of-goods than by degrees of biologically mimicry, which currently separate the resulting tissue engineered corneas.

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