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Corneal tissue engineering: a personal perspective

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There persists a growing and urgent need for engineered corneal tissue. We have taken an early lead on engineering cell-contrived corneal tissue i.e. controlling the inherent ability of corneal stromal cells to self-assemble and produce a collagen rich corneal stromal tissue - a mimic. We have dubbed this *in vitro* approach "tissue-templating". Central to this method is the axiom, 'where cells lead collagen follows'. Within this short article I will briefly outline the journey we have taken, key milestones and what the future may hold for corneal tissue engineering.

Within the field of corneal tissue engineering numerous attempts have been made to construct stromal tissue equivalents and with varying degrees of success, led by the seminal work of May Griffith et al[1] (and reviewed here[2]). These studies have previously focused on creating materials with appropriate levels of transparency, cell compatibility and mechanical strength. Despite the significance of these achievements few have yet addressed a critical quality - a cornea's shape and thereby its optical strength. An incorrectly shaped corneal construct would cause light to be focused either in front or behind the retina and thus the engineering of such a living tissue that can perform this function is, we believe, a key milestone in the development of a tissue engineered cornea.

The approach we have taken to create a functional tissue engineered cornea has been reductionist. We have endeavored, over the last 20 years, to build on the previous work of others and further improve our understanding of the biology of the cornea at a molecular, cellular and tissue level. We have taken this new understanding and looked to apply it in some novel manner in order to improve our tissue engineering of a functional cornea.

For example, we have quantified the nanostructure of the cornea and related this arrangement to the preservation of corneal transparency[3,4], we subsequently applied these measurements to the design of new corneal biomaterials capable of supporting both corneal stromal and epithelial cell self-assembly, differentiation and growth[5]. More recently we have shown how cell-derived hierarchical collagen organisation can be modulated or prescribed by the precise spatio-temporal positioning of corneal stromal cells upon a 2D surface[6,7] (Figure 1). Further studies have shown that by controlling the amount of initial cell alignment collagenous tissues

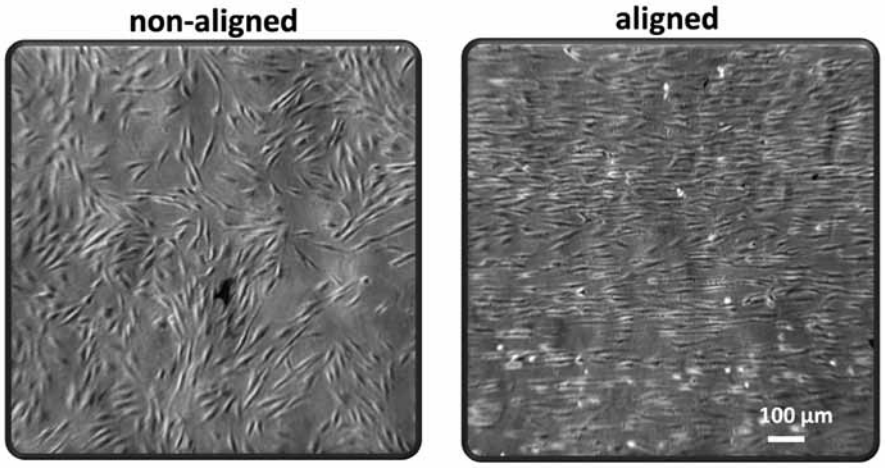

Figure 1. Corneal stromal cells extracted from human limbal tissue and grown in serum free conditions readily respond to an aligned template.

ensue with predictable distinctions in structure and function[8].

This early work strengthened our hypothesis that proper organisation of cells and extracellular matrix (ECM) over multiple length scales is a prerequisite for proper organ function; indeed, within the cornea it is fundamental to its transparency[9]. To address this critical concept tissue engineers have traditionally utilised *top-down* approaches where cells are seeded into artificial[10] or bio-derived scaffolds[11] to provide them with a structural guidance to support the desired tissue organisation and shape. However, we and others have more recently explored the *bottom-up* assembly of cells to induce the formation of tissues with various microengineered geometries, which in turn offers improved function and/or greater control of tissue structure when compared with engineered structures using a top-down approach[8,12,13] (Figure 2).

A bottom-up approach is potentially much more powerful than a top-down approach as it works on the principle that if fully functional tissues can be generated from cell populations during normal development then that these cells can reenact this in vitro if given appropriate growth conditions. Thus, the final tissues have the correct structure and function driven by appropriate nanoscale resolution of the ECM and proper spatial positioning and differentiation of the component cells. We believe that this level of organization is not possible, or at least not commercially scalable, with current top-down approaches to tissue engineering.

We achieve bottom-up or cell derived tissue formation using a cell culture technique we have called "tissue-templating". Our first demonstration of this approach was the use of aligned cell binding motifs patterned on to a glass slide[6]. Growth of corneal stromal cells upon this surface resulted in highly aligned cells. Moreover, these aligned cells then produced similarly aligned fibrillar collagen[7]. Intriguingly as the cells continued to grow and stratify they would do so perpendicular to the layer below and with thick layer of aligned collagen between them. This created tissue with similar hierarchical structures (orthogonal placed layers of bundled collagen fibrils i.e. lamellae) to the tissue from which the cells were initially taken i.e. the

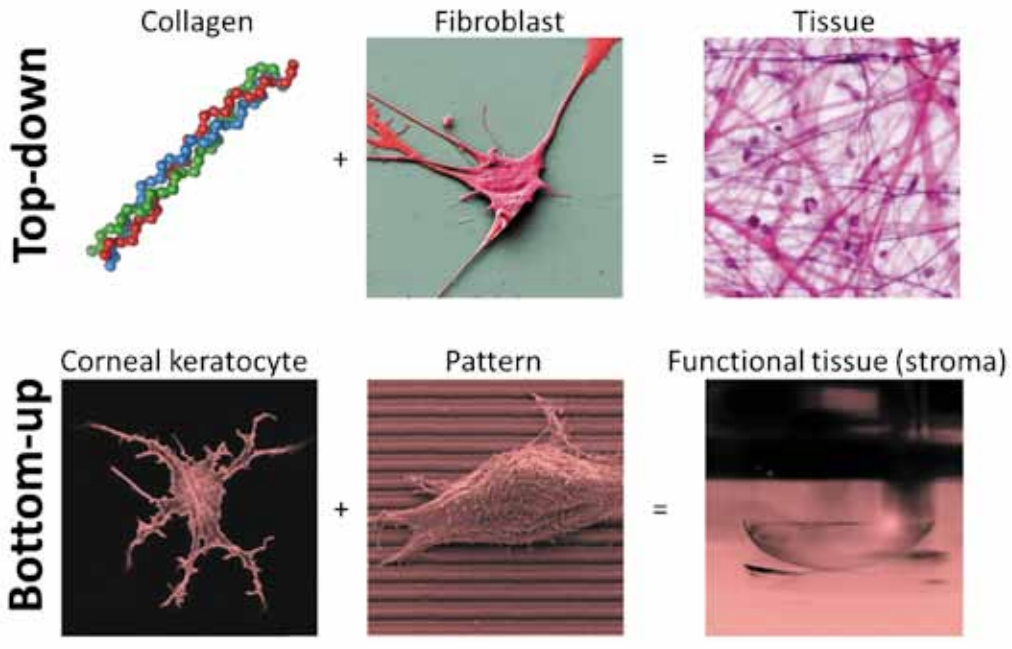

Figure 2. Mixing cells with a material such as collagen often results in a simple tissue, lacking order and proper function (top-down). However, using a phenotypic cell type under specific direction creates highly organized growth and extracellular matrix production resulting in a complex tissue with enhanced functionality (bottom-up).

cornea[8]. An assumption underlying such an approach is that the spatial organisation, initially imposed by artificial environments (template), is basically preserved throughout the tissue remodeling phases.

Whilst much of our earlier studies were performed using 2D templates to drive cell organisation and subsequent tissue organisation, some work has also been carried out using 3D templates. For example, we have shown that template curvature influences cell alignment to create improved human corneal equivalents[14] and that 3D bioprinting of a corneal stroma equivalent is possible[15]. Recent work has also been performed in 4D tissue engineering[16].

Within a 4D approach, the additional dimension is time, thus it is possible to create a template environment for corneal stromal cells that breaks away from the assumption stated above (that templated spatial organization is preserved throughout the process) i.e. that cells change their organization during the culture period and a time resolved tissue ensues. To demonstrate this, we created flat/planar collagen gels comprising stromal cells with a spatially differentiated bio-chemical cue. The localized interaction with these cues brought about regional differences in cell behavior, namely contraction, which resulted in the planar tissue forming a curved corneal-like tissue over 5 days in culture.

Looking forward we believe tissue-templating holds great promise as a scalable means to generate environments for corneal cells to grow from, (i.e. the manufacture of proto-corneas that mature either inside or outside the body) and into functional corneal replacements. Whilst much of our work has been focused on only one part of the cornea, the stroma, we argue that the stroma itself is a natural template upon which the remaining corneal cells can react. For example, we have recently shown that stromal stiffness affects corneal epithelial cell differentiation and that modulating corneal tissue biomechanics can restore limbal stem cell function following pathological stiffening of the stroma (i.e. chemical burn)[17]. Moreover, from a tissue engineering perspective we have shown that it is possible to template stromal cells, within a single construct, to produce an ECM with different and precise levels of stiffness. Such constructs were then shown to support epithelia growth at corresponding levels of differentiation e.g. undifferentiated at the circumference and differentiated at the centre across its surface[16].

In summary, much has been achieved in a relatively short period of time and it is my contention that the cornea, because of its unique physiology, will be one of the first routinely transplanted tissue engineered therapeutic products and that we will see this within the next decade.

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