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Leading Opinion

Silk fibroin in ocular tissue reconstruction

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ABSTRACT

The silk structural protein fibroin displays potential for use in tissue engineering. We present here our opinion of its value as a biomaterial for reconstructing tissues of clinical significance within the human eye. We review the strengths and weaknesses of using fibroin in those parts of the eye that we believe are most amenable to cellular reconstruction, namely the corneoscleral limbus, corneal stroma, corneal endothelium and outer blood-retinal barrier (Ruysch's complex). In these areas we find that by employing the range of manufacturing products afforded by fibroin, relevant structural assemblies can be made for cells expanded *ex vivo*. Significant questions now need to be answered concerning the effect of this biomaterial on the phenotype of key cell types and the biocompatibility of fibroin within the eye. We conclude that fibroin's strength, structural versatility and potential for modification, combined with the relative simplicity of associated manufacturing processes, make fibroin a worthy candidate for further exploration.

1. Introduction

Fibroin is a group of fibrous proteins produced by some members of the classes *Insecta and Arachnida*, generally to make webs or cocoons. The secondary structure of fibroin proteins is responsible for the mechanical properties of silk fibres and has lead to these proteins being considered as biomaterials for tissue engineering and other biomedical applications [1]. The commercial availability of silk cocoons from the domesticated silkworm *Bombyx mori* has focused most studies on this source. Whilst the majority of applications have been considered with respect to reconstructing musculoskeletal and vascular tissues [2–8], a small number of recent studies, including those by our own group, have begun exploring the potential use of fibroin for engineering more functionally complex tissues such as those found within the eye [9–12]. The results from these preliminary studies are encouraging, but there is still much to be learned about optimising fibroin-based materials for biomedical applications. Moreover, strategies for using fibroin within the eye have yet to be fully explored within a clinical context. The purpose of this article is therefore to clarify which tissues within the eye and which diseases stand to benefit most from use of bioengineered tissue. From these analyses we propose clinically appropriate strategies for treatment with fibroin-based materials. While doing so, we highlight current deficiencies in the general understanding of cell–fibroin interactions which in our opinion will need to be addressed to properly exploit these processes for biomedical applications.

2. What types of bioengineered ocular tissue are needed?

The eye like many organs is prone to a variety of pathologic conditions throughout life which can lead to loss of normal tissue structure and function. These losses result in reduced vision, sometimes blindness. In some cases vision can be restored through removal of the diseased tissue and implantation of a medical device. For example, the development of intraocular lenses (IOLs) using various acrylic polymers has transformed the treatment of cataracts to the point where their use can now be considered routine [13]. The artificial cornea or keratoprosthesis synthesized from poly(2-hydroxyethyl methacrylates) (PHEMA) has also proved to be useful as a last resort in cases when conventional surgical options have

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failed [14]. The purpose of this article, however, is to consider those eye conditions for which transplants of bioengineered tissue are being developed as a first-line therapy. These conditions relate to four specific regions within the eye: the corneoscleral limbus, the corneal stroma, the corneal endothelium, and the outer blood-retinal barrier also known as Ruysch's complex. The location and histological structure for each region is shown in Fig. 1.

2.1. Overview of the cornea

The cornea of the eye is a relatively simple structure measuring approximately half a millimetre in thickness and consisting of three distinct tissues: the corneal epithelium consisting of 3–5 layers of stratified squamous epithelial cells residing anterior to an acellular zone known as Bowman's layer (BL), an avascular corneal stroma sparsely populated by a mesenchymal cell type known as the keratocyte, and an innermost single layer of corneal endothelial cells which resides posterior to an acellular zone known as Descemet's membrane (DM) (Fig. 1B). While the smoothness of the epithelium combined with the optical properties of the stroma constitute the primary refractive element in the visual pathway, the endothelial layer is also critical for vision as this single layer of cells regulates the degree of corneal hydration and in doing so maintains the precise spacing of extracellular matrix (ECM) components necessary for a clear cornea.

The corneoscleral limbus is a narrow transitional zone of tissue which separates the clear cornea from the surrounding "white" sclera (Fig. 1A). Across this transitional zone, the surface of the eye changes from corneal epithelium covering the cornea, to conjunctival epithelium which covers the scleral surface. While the stratified squamous epithelial cells of the cornea maintain a smooth ocular surface, the conjunctival epithelium contains mucus-secreting goblet cells which help to protect and lubricate the surface of the eye. Both tissues are maintained throughout adult life *via* the actions of epithelial progenitor cells. Progenitor cells for the corneal epithelium in humans reside predominantly within the basal layer of the limbal epithelium [15], which in some areas can be seen to bulge into the adjacent stroma resulting in the formation of epithelial crypts [16]. The limbal region differs from the cornea by the absence of BL, a more densely populated stromal layer, and the presence of a network of terminating blood vessels. Together these histological features contribute to formation of an epithelial stem cell niche [17].

2.2. Tissue for repairing the corneoscleral limbus

Injuries and diseases affecting the limbus have marked effects on the stability of the ocular surface (Fig. 2B and Fig. 3D). A typical example occurs in the case of chemical burns whereby after limbal damage, subsequent healing of the ocular surface can often only occur *via* ectopic expansion of the peripheral conjunctival epithelium. Since the conjunctival epithelium is relatively opaque and less able to withstand frictional forces associated with normal eyelid movement, vision is significantly impaired and the unstable surface leads to chronic inflammation and scarring within the underlying stroma.

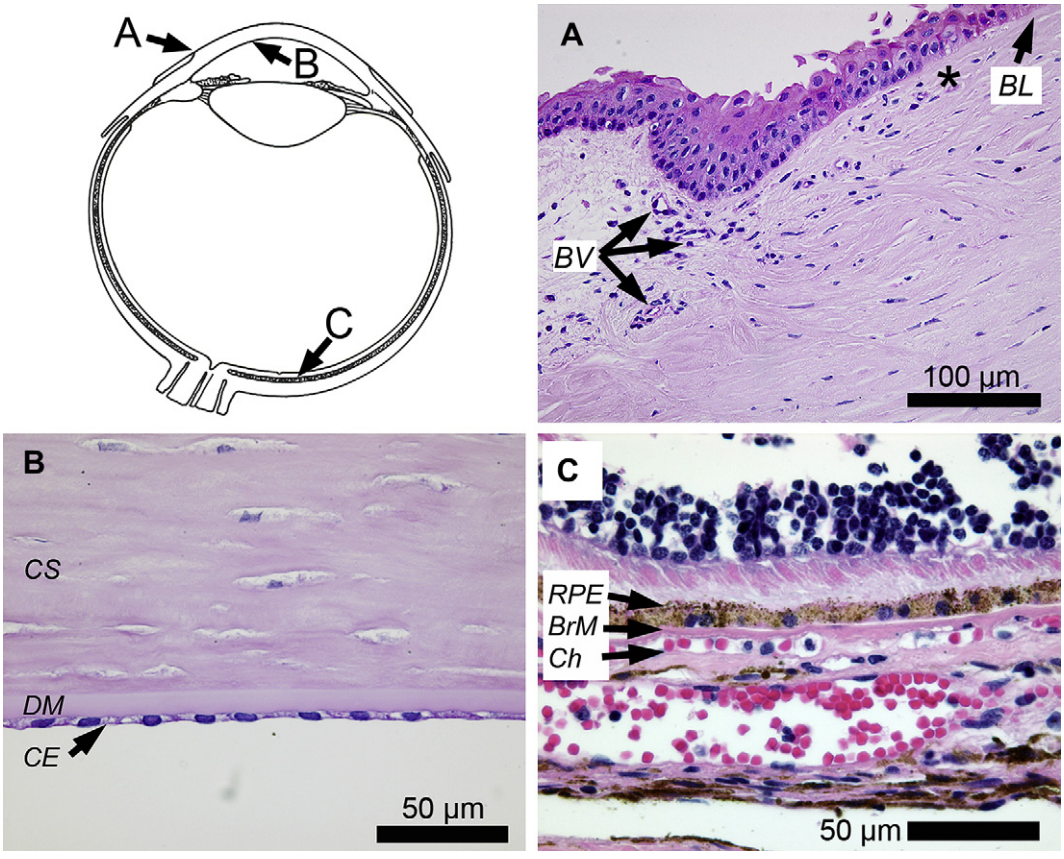

Fig. 1. Regions within the human eye where there is greatest need for bioengineered tissue. (A) Corneoscleral limbus: this transitional zone between the cornea and sclera contains progenitor cells for maintaining the corneal epithelium. The absence of Bowman's layer (*BL*; approximate end point marked with asterisk) and presence of stromal blood vessels (*BV* and arrows) may well contribute to maintenance of this stem cell niche. (B) Corneal stroma (*CS*) and corneal endothelium (*CE*): precise orientation and spacing of ECM components within the stroma provides a clear refractive element essential for vision. The correct spacing of ECM components required for a clear cornea is maintained by the corneal endothelium which regulates hydration of the CS. Descemet's membrane (*DM*) can also be seen. (C) Ruysch's complex: consists of the retinal pigment epithelium (*RPE*), Bruch's membrane (*BrM*) and choriocapillaris (*Ch*). Together these structures nourish and support attachment of the retina to the underlying choroid.