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Corneal structure and transparency

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ABSTRACT

The corneal stroma plays several pivotal roles within the eye. Optically, it is the main refracting lens and thus has to combine almost perfect transmission of visible light with precise shape, in order to focus incoming light. Furthermore, mechanically it has to be extremely tough to protect the inner contents of the eye. These functions are governed by its structure at all hierarchical levels. The basic principles of corneal structure and transparency have been known for some time, but in recent years X-ray scattering and other methods have revealed that the details of this structure are far more complex than previously thought and that the intricacy of the arrangement of the collagenous lamellae provides the shape and the mechanical properties of the tissue. At the molecular level, modern technologies and theoretical modelling have started to explain exactly how the collagen fibrils are arranged within the stromal lamellae and how proteoglycans maintain this ultrastructure. In this review we describe the current state of knowledge about the three-dimensional stromal architecture at the microscopic level, and about the control mechanisms at the nanoscopic level that lead to optical transparency.

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1. Introduction

The transparent cornea forms the anterior portion of the outer casing of the eye and has the dual functions of protecting the inner contents of the eye as well as providing about two thirds of the eye's refractive power. The human cornea is composed of five layers, an overlying epithelium beneath which is a fibrous meshwork called

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Bowman's layer. The bulk of the tissue is constituted by the stroma, a collagen-rich central layer that comprises nearly 90% of the thickness of the cornea, and beneath this lies Descemet's membrane which supports the single layer of endothelial cells lining the posterior cornea. Other species have been reported to have certain differences in this construction, particularly with respect Bowman's layer and Descemet's membrane (Hayashi et al., 2002) but nevertheless, all these corneal layers need to be transparent. In normal corneas most of these are so thin that light scattering is minimal. For example, in humans, Bowman's layer and Descemet's membrane, both collagenous tissues like the stroma, together contribute less than 4% to the total corneal thickness. The corneal epithelium, on the other hand, is about 53 μm deep (Reinstein et al., 2008) and thus constitutes about 10% of the corneal thickness. Its transparency is a result of the homogeneity of the refractive index of all its constituent cells (Dohlman, 1971). In this review, we will concentrate on the structure and transparency of the corneal stroma. However, it should be noted that in a number of corneal pathologies, changes in one or more of the other layers can lead to increased light scattering and consequent loss of corneal transparency.

In most mammals, the cornea is the only tissue requiring considerable tensile strength coupled with a perfectly defined shape and optical clarity. For nearly a century it has been realised that these properties may derive from the arrangement of the constituents of the stroma (Eisler, 1930; Kolmer and Lauber, 1936). About 50 years ago the basic ultrastructure had been described (Jakus, 1962) and the principles behind corneal transparency finally elucidated (Maurice, 1957; Hart and Farrell, 1969; Benedek, 1971) so corneal research focussed more on other areas. However, with the advent of a host of new clinical techniques ranging from laser refractive surgery to corneal cross-linking, interest in how the shape, strength and transparency of the cornea is achieved and maintained has grown. The past decades have seen the emergence of a number of exciting new methodologies that have allowed us to gain considerable insight into how this structure forms, how it is maintained, and how it achieves the biomechanical and optical properties required for a functional cornea. These new discoveries are supporting efforts by surgeons and others to improve clinical techniques, and by bio-engineers and computer modellers to understand and predict the behaviour of the tissue after surgical interventions. They also underpin efforts to develop artificial biological corneal replacements. In this review, we describe our current understanding of the structure of the corneal stroma at all hierarchical levels. We review the latest advances demonstrating how the microscopic structure controls the shape of the cornea, how the nanoscopic arrangement of collagen fibrils ensures corneal transparency, and how this unique fibrillar arrangement arises and is maintained.

2. Stromal micro- and nano-structure

The corneal stroma has three primary non-aqueous constituents: collagens, proteoglycans and cells. It also contains specialised glycoproteins (Labat-Robert and Robert, 2012; Wall et al., 1988; Cooper et al., 2006) and, of course, ions that play an important role in organising the collagen fibrils in order to maintain transparency (Kostyuk et al., 2002; Regini et al., 2004). Many of the characteristics of corneal collagen and its structural organisation have been described elsewhere (Meek and Quantock, 2001; Meek and Boote, 2009; Meek, 2009) so here we give only a brief overview and a more extensive update of the most recent findings.

2.1. Lamellae

It has long been known that, at the microscopic level, the collagen in the stroma is laid down within lamellae. These structures are of variable thickness, in humans typically up to 0.2 mm broad and 2 μm thick (Polack, 1961; Komai and Ushiki, 1991). At the centre of the human cornea there are approximately 200 lamellae through the thickness, and the packing density is higher in the anterior lamella than in the posterior ones (Bergmanson et al., 2005). These anterior lamellae are highly interwoven (Radner et al., 1998) and most appear to insert into Bowman's layer (Morishige et al., 2006). The mid-stromal lamellae are also highly interlaced (Radner and Mallinger, 2002). The posterior lamellae in the central cornea are more hydrated and are believed to have less interlacing, lying on top of each other like the layers in plywood. The posterior stroma can swell easily whereas the more interwoven anterior cannot (Müller et al., 2001).

With the advent of new corneal surgical techniques aimed at avoiding penetrating keratoplasty (such as Deep Anterior Lamellar Keratoplasty, Descemet's Stripping Endothelial Keratoplasty and related procedures) it is becoming common to inject air into the cornea to separate the endothelium and Descemet's membrane (pneumodissection) in order to make the surgery easier to perform. For some time, ophthalmologists have been aware that when a so-called “big bubble” is induced, part of the posterior stroma often adheres to the Descemet's membrane, and that there is, therefore, a natural cleavage plane in the stroma about 10 μm above Descemet's membrane (Jafarinasab et al., 2010; McKee et al., 2011). A study of the different types of big bubble that can be formed led Dua et al. (2013) to propose, somewhat controversially, that the most posterior region of the stroma below the final layer of keratocytes is distinct, and should therefore be classed as a separate layer, which they termed Dua's layer or pre-Descemet's layer (PDL). This region was shown to be intimately related to the trabecular meshwork and, like that structure, to be rich in type VI collagen (Dua et al., 2014). Barring an anchoring zone of interwoven collagen fibrils at the Descemet-stroma interface, Schlötzer-Schrehardt et al. (2015) found no evidence for the existence of a distinctive acellular PDL in the human cornea. They concluded that the intrastromal cleavage plane after pneumodissection was a result of the specific arrangement of keratocytes across the cornea in this region, and that it is determined by the intra-individually and inter-individually variable distances of keratocytes from Descemet's membrane. Although our own observations of this region, using X-ray scattering, have revealed no differences in the collagen fibrillar arrangement, there is also an elastic fibre network within the stroma (McIlroy, 1906; Alexander and Garner, 1983). We have shown in the human cornea that these elastic fibres, though present throughout most of the stromal depth, are concentrated below the posterior-most keratocyte layer (unpublished results). Notwithstanding these findings, at the time of writing the jury is still out, although it cannot be denied that the way injected air moves through the stromal lamellae in this region has very important implications for big bubble surgical techniques.

Moving from the central cornea towards the limbus the human cornea thickens; hydration is fairly constant in this direction in the pig and the cow (S. Hayes, unpublished results; Ho et al., 2014) and, although water distribution from central cornea to limbus is not known for human corneas, it seems likely that alterations in tissue thickness are due to an increase in the amount of collagen in the peripheral stroma (Aghamohammadzadeh et al., 2004; Boote et al., 2011; Henriksson et al., 2012). In addition, stromal interweaving in the peripheral cornea seems to extend to the deeper posterior lamellae (Radner et al., 1998; Abass et al., 2015). The posterior limbus accommodates a circum-corneal annulus (Fig. 1a) in which elastic fibres run parallel to collagen fibres (Kamma-Lorger et al., 2010). The exact arrangement of this elastic system in the rest of the cornea remains to be elucidated, but current work in our group has revealed that many fibres seem to originate in the limbus,