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Review

Regulation of corneal stroma extracellular matrix assembly☆

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ABSTRACT

The transparent cornea is the major refractive element of the eye. A finely controlled assembly of the stromal extracellular matrix is critical to corneal function, as well as in establishing the appropriate mechanical stability required to maintain corneal shape and curvature. In the stroma, homogeneous, small diameter collagen fibrils, regularly packed with a highly ordered hierarchical organization, are essential for function. This review focuses on corneal stroma assembly and the regulation of collagen fibrillogenesis. Corneal collagen fibrillogenesis involves multiple molecules interacting in sequential steps, as well as interactions between keratocytes and stroma matrix components. The stroma has the highest collagen V:I ratio in the body. Collagen V regulates the nucleation of protofibril assembly, thus controlling the number of fibrils and assembly of smaller diameter fibrils in the stroma. The corneal stroma is also enriched in small leucine-rich proteoglycans (SLRPs) that cooperate in a temporal and spatial manner to regulate linear and lateral collagen fibril growth. In addition, the fibril-associated collagens (FACITs) such as collagen XII and collagen XIV have roles in the regulation of fibril packing and inter-lamellar interactions. A communicating keratocyte network contributes to the overall and long-range regulation of stromal extracellular matrix assembly, by creating micro-domains where the sequential steps in stromal matrix assembly are controlled. Keratocytes control the synthesis of extracellular matrix components, which interact with the keratocytes dynamically to coordinate the regulatory steps into a cohesive process. Mutations or deficiencies in stromal regulatory molecules result in altered interactions and deficiencies in both transparency and refraction, leading to corneal stroma pathobiology such as stromal dystrophies, cornea plana and keratoconus.

1. Introduction

The cornea is the transparent tissue at the anterior of the eye. Transparency is its main function, allowing the passage of light, and the cornea is the major refractive structure of the eye. The cornea, together with the sclera, also provides mechanical stability and a protective barrier. The cornea is composed of three layers: an outer epithelial layer; a middle layer – the stroma that is the focus of this review, and an inner endothelial layer. The corneal stroma comprises about 90% of the thickness of the cornea. It is a collagen-rich extracellular matrix (ECM) assembled to provide transparency and maintain the structure required for refraction of light. The main structural features of the stroma are dense, regularly packed collagen fibrils. The collagen fibrils have a homogeneous distribution of small (~25 nm) diameters. The fibrils are organized as regularly packed bundles or fibers. The fibers coalesce and form stacked layers or lamellae (Fig. 1). The organization of lamellae has been and continues to be described as orthogonal which indicates a 90° offset of adjacent lamellae. Individual sections support a description of adjacent lamellae being offset roughly 90° from each other. However, inter-lamellar angles ranging from 1° to 90° have been found associated with different species and stromal locations (Radner et al., 1998; Thomasy et al., 2014). Therefore, the over generalized used of “orthogonal” misrepresents structural differences that are undoubtedly critical determinants of functional requirements. However, the majority of lamellae are offset from adjacent lamellae forming a plywood-like structure and terms such as ‘roughly orthogonal’ are likely to be perpetuated even as work detailing the structure–function relationships of this level of the stromal hierarchy continues. Finally, based on the physical properties of light, the regular, stacked lamellar structure of the corneal stroma produces minimal light scattering and therefore maximal

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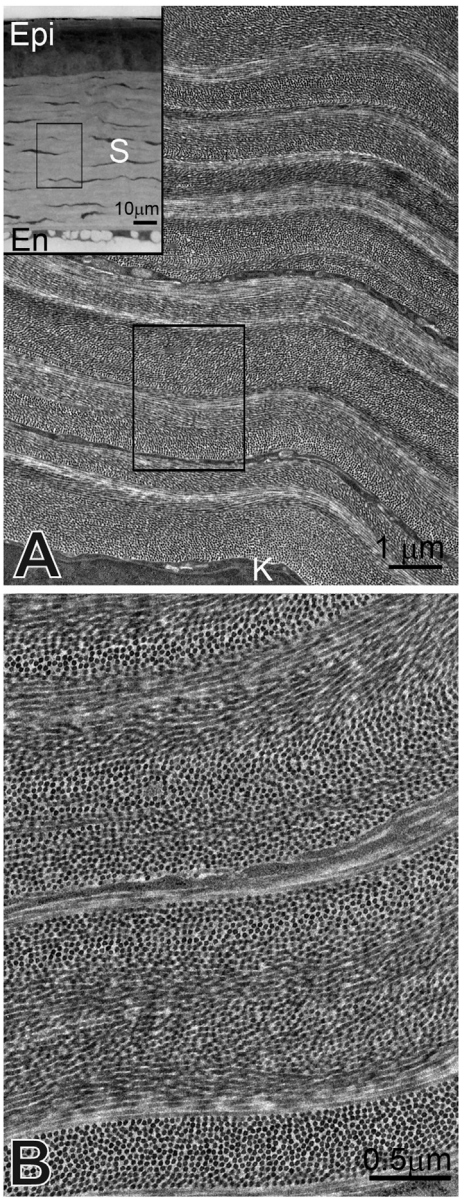

Fig. 1. The ultrastructure of the cornea stroma. Three layers specifically define the cornea: the outer epithelium, the stroma, and the inner endothelium. All three layers can be seen; the stroma comprises more than 90% of the corneal thickness **(A, inset)**. **(A)** The stroma contains keratocytes (K) positioned parallel to the corneal surface, between the stromal lamellae. **(B)** The lamellae are composed of small diameter collagen fibrils with regular packing; adjacent layers are at approximately right angles to one another (B, is an enlarged area of the rectangle in A). P30 mouse cornea; A, inset light micrograph; A and B transmission electron micrographs. Figure modified from Hassell and Birk (2010).

transparency (Benedek, 1971; Farrell et al., 1973; Maurice, 1957). The structural features provide the molecular basis required for stromal transparency; therefore, regulation of stromal extracellular matrix assembly is essential for corneal transparency.

The cornea has a different curvature from the rest of the ocular surface. In addition, there is a difference in the thickness between the central and peripheral cornea. In the central cornea, the majority of collagen fibrils are oriented superior–inferior or nasal–temporal, orthogonally. At the periphery, however, collagen fibril bundles are branched and interlacing gradually into a circular orientation at the cornea and sclera interface (Boote et al., 2008). There are also differences in fibril organization between anterior and posterior stroma in the nasal and temporal regions. These regional differences in collagen fibril organization provide mechanical strength and underlie the structural features critical for maintaining corneal shape and its refractive properties.

Extracellular matrix assembly in the corneal stroma is tightly regulated during development. This review will focus on the regulation of stromal extracellular matrix assembly during development and maintenance of stromal structure. However, the concepts discussed will provide a foundation for studies of corneal stromal pathophysiology, as well as application to the corneal stroma of the approaches utilized in regenerative medicine.

2. Corneal stromal assembly during development

The development of the corneal stroma has been extensively studied in the chicken (Cornuet et al., 1994; Coulombre and Coulmbre, 1958b; Coulombre and Coulombre, 1958a; Funderburgh et al., 1986; Hay, 1977; Hay and Revel, 1969; Quantock and Young, 2008; Toole and Trelstad, 1971) and the rabbit (Cintron and Covington, 1990; Cintron et al., 1988a; Cintron et al., 1984; Cintron and Hong, 1988; Cintron et al., 1988b; Takahashi et al., 1993; Zhan et al., 1995). In avian species, neural crest cells migrate into the space between the corneal epithelium and the lens epithelium, and they are loosely aggregated into several layers filling the space. The first wave of neural crest cells condenses to form the corneal endothelium. Additional neural crest cells between the corneal epithelium and endothelium become keratoblasts. In avian development, corneal epithelial cells secrete a structured acellular matrix as a rudimentary primary stroma, before the neural crest cells that will form keratoblasts migrate into the stroma. There are numerous variations in stromal development between different species. In mammalian species there is no primary stroma, but overall the two waves of neural crest invasion, endothelial and keratoblast are comparable (Hay, 1980).

Keratoblasts are the major cells that synthesize the molecular components of the embryonic corneal stroma. They continue to proliferate, and differentiate into keratocytes. Keratocytes proliferate slower, but synthesize high levels of collagens and proteoglycans (Cintron et al., 1983; Cornuet et al., 1994; Funderburgh et al., 1986; Young et al., 2007). As corneal stromal development progresses, the keratocytes maintain long-range associations with assembled collagen fibrils through an extended network of cytoplasmic filopodia. These cellular protrusions/processes are distinct and common during the course of stromal development and particularly lamella formation (Birk and Trelstad, 1984; Young et al., 2014). Corneal development continues in the postnatal period, yielding complete transparency (Chakravarti et al., 2006; Coulombre and Coulmbre, 1958b; Coulombre and Coulombre, 1958a; Song et al., 2003). Analysis of stromal development in embryonic chickens demonstrated that collagen fibrillogenesis occurred within small surface recesses of the keratocytes (Birk and Trelstad, 1984). Folds in the cell surface continued to fuse as the cell surface retracted, thus forming large surface-associated compartments in which fibril bundles (fibers) coalesced to form lamellae (Fig. 2). During stromal development, keratocytes and their processes have two major axes at approximately right angles to one another (Fig. 3). The surface compartments involved in the production of the corneal stroma are aligned along two axes that are ~90° to one another. Thus, the cells are capable of forming ECM with fibril bundles heading in two directions perpendicularly. Using a 3-D reconstruction technique, it has been demonstrated that by volume, keratocytes occupy more than 20% of the stroma, and the filopodia of the keratocytes can extend more than 30 μm into the extracellular space. Moreover, there is a clear orthogonal organization in both cell and matrix organization (Young et al., 2014).