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Cell regulation of collagen fibril macrostructure during corneal morphogenesis

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ABSTRACT

While tissue form and function is highly dependent upon tissue-specific collagen composition and organization, little is known of the mechanisms controlling the bundling of collagen fibrils into fibers and larger structural designs that lead to the formation of bones, tendons and other tissues. Using the cornea as a model system, our previous 3 dimensional mapping of collagen fiber organization has demonstrated that macrostructural organization of collagen fibers involving interweaving, branching and anastomosing plays a critical role in controlling mechanical stiffness, corneal shape and refractive power. In this work, the cellular and mechanical mechanisms regulating critical events in the assembly of collagen macrostructure are analysed in the developing chicken cornea. We elucidated the temporal events leading to adult corneal structure and determined the effects of intraocular pressure (IOP) on the organization of the collagen macrostructure. Our findings indicate that the complex adult collagen organization begins to appear on embryonic day 10 (E10) after deposition of the primary stroma and full invasion of keratocytes. Importantly, organizational changes in keratocytes appearing at E9 preceded and predicted later changes in collagen organization. Corneal collagen organization remained unaffected when the development of IOP was blocked at E4. These findings support a primary role for keratocytes in controlling stromal organization, mechanical stiffness and corneal shape that are not regulated by the IOP. Our findings also suggest that the avian cornea represents an excellent experimental model for elucidating key regulatory steps and mechanisms controlling the collagen fiber organization that is critical to determining tissue form and function.

Statement of significance

This work by using an ex ovo model system, begins to investigate the potential mechanisms controlling collagen fibril macrostructure. In particular, this work highlights a convergent role for the corneal keratocytes in organizing the complex collagen macrostructure, necessary to support high visual acuity. Our data supports that the intraocular pressure does not influence collagen fibril macrostructure and suggest that the avian cornea represents an excellent experimental model for elucidating key regulatory steps and mechanisms controlling the collagen fiber organization that is critical to determining tissue form and function. Clearly understanding the cellular and molecular mechanisms that underlie collagen fibril macrostructure will be highly beneficial for future tissue engineering and regenerative medicine applications.

1. Introduction

While the mechanisms regulating the tissue-specific assembly of collagen fibril diameter and spacing are well established [1–5], collagen fibrils are only the basic building blocks for connective

Abbreviations: 3D, Three-dimensionally; E, Embryonic Day; FFT, Fast Fourier Transform; IOP, Intraocular Pressure; SHG, Second Harmonic Generation.

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tissues, and require bundling to form groups of fibrils forming fibers that are organized into hierarchical structures in a tissue-specific fashion that dictate tissue form and function, such as the coaxially aligned bundles of collagen fibres in tendon [6–8], the interwoven laminated structure in intervertebral disc [9] and the orthogonally arranged and intertwined collagen lamellae of the cornea [10–15]. Less is known with respect to the temporal and spatial mechanisms (cellular, molecular and mechanical) that regulate the assembly of these hierarchal collagen structures and as stated by Trelstad and Birk in 1984, "the weaving of the body fabric from the warp and woof of the matrix has yet to told [16]."

Specifically, the highly ordered collagen architecture of the corneal stroma provides a model system to unravel these mechanisms and key regulatory steps involved in the collagen macrostructure and tissue architecture required for function. The cornea of the vertebrate eye plays a critical role in vision, serving both as a transparent window and a refractive lens that provides over two thirds of the refractive power of the eye in mammals. The principal parts of the cornea are the corneal epithelium, the corneal endothelium and the corneal stroma, which is comprised predominantly of collagen, making up 90% of the corneal thickness. Using non-linear second harmonic generation (SHG) imaging to 3 dimensionally (3D) map the collagen fiber/lamellar architecture, we have previously shown that there are progressive changes within the macrostructure of the cornea from extant species of different vertebrate clades suggesting evolutionary adaptations to accommodate control of corneal shape and refractive power involving increasing lamellar complexity through branching and anastomosing [10,11].

Although the cellular, molecular and mechanical mechanisms controlling these evolutionarily adaptations remain unknown, the patterning of the corneal stroma is likely determined during embryonic development with variations in stromal organization dependent on the vertebrate species. Tissue morphogenesis is a fundamental developmental process closely coupled with a series of matrix-matrix, matrix-cell and cell-cell interactions [1,17–21]. In like manner, corneal morphogenesis [22–24] is characterized by a series of morphological and biochemical events that take place as the cornea develops, giving the tissue its unique shape, biomechanical properties, structure and composition [25–31].

In particular, chick corneal morphogenesis has been studied extensively and shown to be dependent on the deposition of an acellular primary stroma synthesized by the corneal epithelium [23,32–36] prior to the inward migration of neural crest cells and the deposition of a secondary stroma by the corneal keratocyte [23,31,33–34,37]. Overall, the consensus view is that the acellular primary stroma serves as a scaffold or template for the production of the secondary corneal stroma by the invading neural crest and that the primary stromal architecture mirrors the adult stromal architecture [23,31,38–39].

Recently, developmental studies of the chick cornea using serial block face scanning electron microscopy have identified keratocyte processes, termed keratopodia, that show synchronized alignment with depositing collagen matrix [40]. In a similar manner to

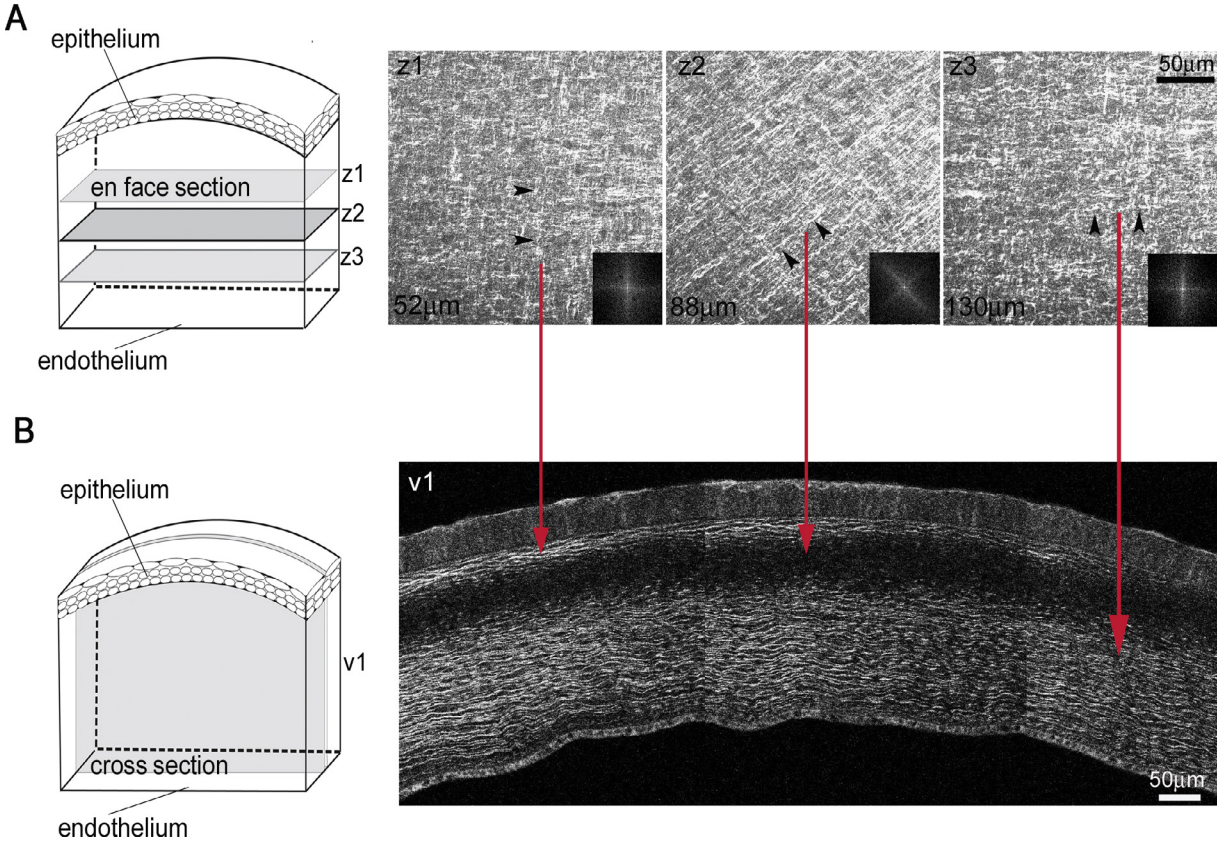

Fig. 1. . An orthogonal/rotated collagen fibril pattern in the chick corneal stroma. (A) Representative en block tissue SHG imaging of collagen macrostructure in the embryonic chick corneal stroma (n = 4). Through focus SHG images taken from the epithelial layer towards the endothelial layer of E17 chick corneal stroma and their corresponding FFT analysis (insets) of the collagen macrosctructure orientation. Black arrowheads denote collagen orientation in the different planes, z1–z3. Scale bar: 50 µm (B) Corneal cross-sections were taken through the entire cornea including the corneal epithelium, stroma and endothelium. SHG signals were only detected when collagen fibers are aligned along the imaging plane and appear as bands in the anterior and posterior stroma. Red lines connect the collagen orientation between the en face (A) and cross-section (B) views. Note that when the collagen is oriented 45° to the image plane, there is no signal (representative plane, z2). Scale bar: 50 µm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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