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# Three-dimensional reconstruction of collagen–proteoglycan interactions in the mouse corneal stroma by electron tomography

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ABSTRACT

Corneal transparency is fundamental to the visual system, and is directly related to the ordered collagen fibril architecture that the cornea maintains. Proteoglycans, through their protein core and highly anionic glycosaminoglycan side chains, are thought to regulate the collagen organisation in the corneal stroma. To understand the inter-relationships between proteoglycans and collagen fibrils in the cornea, adult mouse corneas were treated with cuprolinic blue and three-dimensional reconstructions of the anterior, mid and posterior corneal stroma were obtained. The reconstructions show regular diameters of collagen fibrils throughout the cornea and uniform interfibrillar spacing within each region. Both longitudinal and transverse reconstructions were obtained to establish a clear picture of proteoglycan organisation, yet no distinct regular pattern or symmetry of proteoglycan orientation was observed. Large, electron-dense proteoglycans (possibly chondroitin sulphate/dermatan sulphate proteoglycans) interconnecting two or often three adjacent collagen fibrils are seen, whilst another sub-population of smaller proteoglycans (of the keratan sulphate variety) interconnect only neighbouring fibrils. The reconstructions suggest a complex interaction between proteoglycans and collagen, which allows for the dynamic control of collagen fibril architecture in the cornea.



## 1. Introduction

The cornea is the transparent connective tissue at the ocular surface which is responsible for the refraction of light entering the eye. In the corneal stroma, heterotypic type I/V collagen fibrils, which exhibit uniform diameter and spatial order, are embedded in a matrix of proteoglycans which form a compression-resistant hydrated bio-gel. The regular pseudo-hexagonal organisation of collagen fibrils is essential for corneal transparency (Maurice, 1957; Hart and Farrell, 1969; Benedek, 1971; Farrell et al., 1973) and is believed to be strongly influenced by the hydrated, interfibrillar proteoglycans (Bettelheim and Plessy, 1975; Borcherding et al., 1975; Bettelheim and Goetz, 1976; Castoro et al., 1988).

A corneal proteoglycan is composed of a small leucine rich-repeat protein core, covalently linked to one or more glycosaminoglycan side chains. It is thought that the protein core attaches to collagen fibrils at axially designated sites ('a' and 'c' for keratan sulphate proteoglycans and 'd' and 'e' for chondroitin/dermatan sulphate proteoglycans (Scott and Haigh, 1985, 1988b; Meek et al., 1986)) through non-covalent interactions at the leucine rich region situated on the concave surface of the protein. The glycosaminoglycan component of the proteoglycan is bound covalently to the protein and extends into the interfibrillar space (Iozzo, 1997). Glycosaminoglycans are linear chains of repeating disaccharides that occupy a large volume of space and exhibit strong swelling pressure at relatively low concentrations (Scott, 1995). There are two types of glycosaminoglycan chains in the cornea: keratan sulphate and a hybrid of chondroitin sulphate/dermatan sulphate. Sulphation of the amino sugars in the glycosaminoglycan chains confers a high negative charge to the unbranched molecule and influences both stromal hydration and interactions with other proteoglycans, both of which have bearings on collagen architecture (Bettelheim and Plessy, 1975; Scott, 2001). In the cornea, chondroitin sulphate/dermatan sulphate is attached to decorin and biglycan, whereas keratan sulphate is linked to lumican, keratocan and mimecan (Iozzo, 1997). Biglycan is primarily involved in the early development of the mouse corneal stroma, whereas decorin, which is linked to only one glycosaminoglycan chain, is prevalent in the mouse stroma throughout maturity (Zhang et al., 2009). Proteoglycan-knockout mice have illustrated that proteoglycans are essential components of organised collagen fibril assembly in the cornea (Chakravarti et al., 2000; Quantock et al., 2001; Meek et al., 2003; Liu et al., 2003; Hayashida et al., 2006). The ordered structure of the corneal stroma allows for light transmission and is therefore subject to relatively strict positional regulation of its components, in contrast to the highly tensile, fibrous tendon or the opaque sclera, which contain comparatively fewer proteoglycans (Scott, 1995).

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As mentioned, the small diameters of collagen fibrils and uniform interfibrillar spacing are essential to the physical basis of corneal transparency. This is exemplified by the remarkable regularity of corneal collagen fibril diameter across a wide range of species (Craig and Parry, 1981; Craig et al., 1986). Type V collagen limits lateral aggregation of collagen (Birk et al., 1990) and can therefore govern fibril diameter, as can proteoglycans with or without their glycosaminoglycan chains (Rada et al., 1993). How proteoglycans regulate interfibrillar spacing is less well understood. Several models have been proposed to explain the structural interactions between proteoglycans and collagen in the cornea (Maurice, 1962; Farrell and Hart, 1969; Muller et al., 2004), and a sixfold arrangement of proteoglycans around collagen fibrils has been proposed. However, new evidence suggests that a systematic sixfold arrangement of proteoglycans is not present, and it is postulated that the proteoglycans govern interfibrillar spacing through thermal motion and a control of osmotic pressure instead (Lewis et al., 2010; Knupp et al., 2010). It is possible to determine the spatial distribution of fibrils in the corneal stroma by calculating their radial distribution function, as has been done for human (Connon et al., 2003) and rabbit corneas (Connon et al., 2000). Short-range order of neighbouring fibrils has been shown to correspond with the onset of transparency and proper formation of the developing avian cornea (Connon et al., 2004), and by determining the radial distribution function for fibrils in the mouse corneal stroma, it is possible to ascertain whether order extends beyond next-nearest neighbours.

The mouse corneal extracellular matrix differs from other mammalian corneas because its proteoglycans are distinctly undersulphated, and a significant proportion of its chondroitin sulphate/dermatan sulphate is present as dermatan sulphate. It also has the highest ratio of idunorate/glucuronate disaccharides within dermatan sulphate (Scott and Bosworth, 1990). Only 18% of the total glycosaminoglycan content within the mouse corneal stroma is present as keratan sulphate (Scott and Bosworth, 1990), significantly lower than in the thicker corneas of larger mammals. Biosynthesis of chondroitin sulphate/dermatan sulphate requires oxygen for glucuronate production, whereas no oxygen is consumed in the synthesis of keratan sulphate from glucose (Stockwell and Scott, 1965). As a consequence, the production of chondroitin sulphate/dermatan sulphate is likely to be preferentially facilitated by the availability of oxygen by diffusion from the atmosphere into the thin mouse corneal stroma. The mouse corneal stroma has a thickness of 60 μm when measured from histological sections (Hayashida et al., 2006) and is approximately 80 μm thick in 3-month old mice when measured by confocal microscopy through focusing (Song et al., 2003). Central corneal thickness, which includes both the epithelium and stroma, has recently been shown to vary considerably across inbred murine species, ranging from 89 to 124 μm with a marked increase in the number of lamellae in the thicker mouse corneas (Lively et al., 2010).

In the present study, we utilised three-dimensional reconstructions of the mouse cornea in order to gain a better understanding of collagen–proteoglycan organisation in the stroma. From structural information revealed through electron tomography we suggest mechanisms through which the organisation of collagen architecture and regulation of interfibrillar spacing by proteoglycans might be maintained.

## 2. Materials and methods

### 2.1. Specimens

Mature, wild-type CD1 mice were obtained from breeding colonies, and eyes were removed within 5 min post-mortem after carbon dioxide asphyxiation and cervical dislocation. Corneas were immediately excised for fixation and staining. Animals were handled in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and local regulations at all times.

### 2.2. Specimen preparation

Corneas were cut into halves with a scalpel and fixed for 24 h in 2.5% glutaraldehyde in 25 mM sodium acetate buffer (pH 5.7) with 0.1 M $MgCl_2$ and 0.05% cuprolinic blue (quinolinic phthalocyanate), a cationic dye for characterisation of proteoglycans (Scott, 1972). Cuprolinic blue complexes with the glycosaminoglycan chains of proteoglycans owing to the presence of negatively charged sulphate and carboxylate residues. After fixation and staining, the samples were washed with sodium acetate buffer followed by aqueous, then 50% ethanolic, sodium tungstate at 0.5%. The samples were dehydrated using an incremental ethanol series and embedded in Araldite resin. Ninety nanometre sections (gold interference) were cut using glass knives and collected on copper slot grids coated with a support film made using 0.38% polyetherimide (PEI) in ethylene dichloride solution. Sections were contrasted with saturated aqueous uranyl acetate solution and 1% aqueous phosphotungstic acid. Finally, grids were exposed to a solution of 10 nm colloidal gold (BBI, Cardiff, UK) to provide gold particles on each face of the slot grid as fiducial markers to be used in the image alignment process.

### 2.3. Electron tomography

Collagen lamellae in each stromal region were imaged via a tilt series ranging from −60° to +60° captured at a magnification of ×20,000, in one degree increments, using a Gatan ORIUS SC1000 CCD camera linked to a JEOL 1010 transmission electron microscope operating at 80 kV. The images captured were then aligned using the IMOD software package (Kremer et al., 1996). Once the images had been carefully aligned according to the positions of individual gold marker fiducials, back-projection and tomogram generation was carried out using the EM3D program (Ress et al., 2004). Segmentation of the three-dimensional reconstruction was also carried out using EM3D.

### 2.4. Radial distribution function

The spatial distribution of collagen fibrils in the mouse cornea was assessed by determining the radial distribution function of fibrils ($n$ = 11,201) throughout the stroma (Cox et al., 1970). A collagen fibril is selected as a reference and the distances of the surrounding fibrils are measured using ImageJ (Abramoff et al., 2004). The frequency plot of the distances of neighbouring fibrils with respect to the reference fibril is then obtained using in-house software. This plot is normalised to account for the fact that the number of fibrils per unit area diminishes as the distance from the reference fibril increases. All fibrils are then utilised in turn, and all frequency plots are summed together in order to improve statistics. Because of the way it is constructed, the radial distribution function describes the lateral packing of collagen fibrils and indicates whether long and/or short-range order exists within a system (Eikenberry et al., 1982).

### 2.5. Measurement of collagen fibril and proteoglycan dimensions

Measurements of collagen fibril and proteoglycan dimensions were carried out using ImageJ software after internal calibration using the collagen D-period (~65 nm in cornea). For the measurement of fibril diameters, 142 collagen fibrils were used. The measurement of proteoglycan length and thickness was limited to

those proteoglycans entirely contained within a plastic section ($n = 50$), as shown by their three-dimensional reconstruction.

## 3. Results

Electron micrographs of the prepupillary region showed two prominent populations of cuprolinic blue-positive proteoglycans and regular collagen fibril order in the anterior (fibril diameters: 30.8 ± 1.9 nm), mid (fibril diameters: 32.0 ± 2.8 nm) and posterior (fibril diameters: 28.9 ± 1.7 nm) mouse corneal stroma (Fig. 1). In between the collagen fibrils, small filament-like structures were numerous (arrows in Fig. 1), as were larger, curved, electron-dense complexes (arrowheads in Fig. 1). These results confirm what was seen in previous electron microscopy investigations of the mouse cornea in two dimensions (Scott and Haigh, 1988a; Young et al., 2005; Hayashida et al., 2006).

Three-dimensional reconstructions of collagen fibrils and associated proteoglycans in longitudinal and transverse sections in the anterior, mid and posterior stroma in the central, prepupillary region of the wild-type mouse cornea were obtained (Fig. 2). The reconstructions of each stromal region showed similar collagen fibril organisation, in which fibrils were of a regular average diameter throughout the stroma and the interfibrillar spacing between fibrils was consistently uniform. No distinct structural differences in proteoglycan content, at the resolution obtained, were observed in the reconstructions of the anterior, mid, or posterior stroma and the distribution of proteoglycan types was also relatively similar. A scatter plot of proteoglycan length versus thickness revealed three morphologically distinct populations throughout the corneal stroma (Fig. 4). Small proteoglycans (31.4 ± 3.5 nm in length measured over the three regions; green arrowheads in Fig. 2a and b) were seen interconnecting only neighbouring fibrils, and were frequently positioned orthogonal to the fibril. Lengths were approximately equivalent to the surface separation of collagen fibrils. Larger proteoglycans varied in thickness, had sinuous profiles and were of two distinct lengths; intermediate sized proteoglycans (64.5 ± 3.9 nm long; orange arrowheads in Fig. 2a and b) usually interconnected two fibrils, but sometimes could be seen to run alongside a collagen fibril, whereas the largest proteoglycans linked three fibrils (122.5 ± 9.8 nm long; red arrowheads in Fig. 2a and b) and were seen less frequently than the other types.

The radial distribution function in Fig. 3 shows that localised, short-range order exists in the mouse corneal stroma, whereas long-range order beyond next-nearest neighbours is not apparent. The function $g(r)$ indicates the probability of locating two fibril centres by a distance $r$. Nearest neighbour separation is illustrated by a prominent first peak at a ~44 nm distance and a more diffuse, less prominent second peak at ~90 nm suggests that next-nearest fibrils show some slight positional regularity. Beyond the second peak, there is little appreciable evidence of positional order between fibrils.

In the reconstructions, proteoglycans showed no specific symmetrical arrangement around collagen fibrils. Neighbouring fibrils were frequently connected at near-regular axial distances, primarily by the small proteoglycans with up to three collagen fibrils often interconnected by the largest proteoglycans.

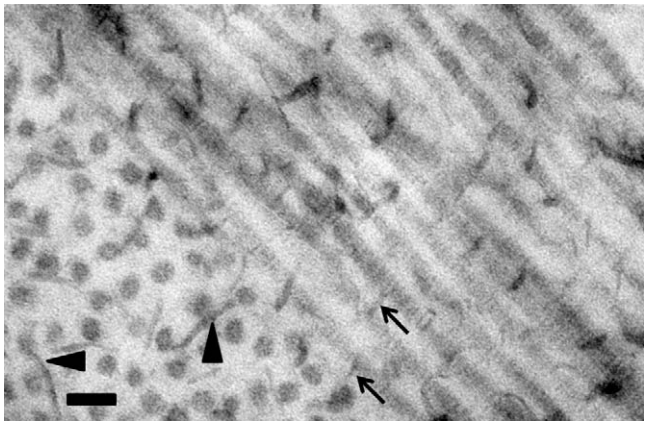

**Fig. 1.** Ultrastructure of the mouse corneal stroma. Two proteoglycan populations are evident in the micrograph. Small, poorly contrasted proteoglycans (arrows) are frequently positioned orthogonally to collagen fibrils whereas the larger, electron-dense complexes (arrowheads) exhibit sinuous profiles. Scale bar = 100 nm.

## 4. Discussion

Our reconstructions provide an insight into the interactions between the structural components of the mouse corneal stroma, illustrating the highly organised collagen fibril architecture and the less well organised three-dimensional arrangement of proteoglycans. A complex, almost random relationship between proteoglycans and collagen is observed, in which adjacent and next-nearest neighbour collagen fibrils are connected by proteoglycans which ultimately are likely to hold the fibril pseudo-lattice arrangement in a three-dimensional matrix.

When interpreting the results presented here, particular attention should be paid to the fact that mouse cornea contains considerable amounts of low-sulphated or unsulphated keratan sulphate (Scott and Bosworth, 1990; Young et al., 2005). Thus, not all keratan sulphate associated proteoglycans may be observed owing to the lower affinity of undersulphated proteoglycans for the cationic cuprolinic blue dye. Chondroitinase ABC digestion of mouse cornea, which cleaves chondroitin sulphate/dermatan sulphate associated glycosaminoglycans, reveals a low population of small, poorly contrasted filamentous structures considered to be keratan sulphate (Young et al., 2005; Hayashida et al., 2006). Keratan sulphate proteoglycans are observed bridging neighbouring fibrils at regular, frequently orthogonal intervals. Chondroitin sulphate/dermatan sulphate proteoglycans on the other hand interconnect neighbours and also next-nearest neighbours, and in some cases lie along the fibril axis.

To accurately determine the morphometry of proteoglycans in mouse cornea, the dimensions of proteoglycans which were wholly contained within the plastic section were measured. The variation in length of chondroitin sulphate/dermatan sulphate proteoglycans suggests a possible end-to-end aggregation (Figs. 5 and 6). There are numerous potential mechanisms that may make this possible, for example, hydrophobic attraction and hydrogen bonding could enable the highly anionic glycosaminoglycans to associate (Scott, 2001), whilst positive ions (i.e. $Na^+$ and $K^+$) drawn into the stroma could screen negative charges and negate the chains' mutual repulsion. An increase in ionic strength has been shown to disrupt aggregation of proteoglycans (Roughley et al., 1995), thus ionic balance might be important for self-association. The observed differences in thickness of our stained proteoglycan filaments (Fig. 4) could be accounted for by variations in sulphation, resulting in a greater accumulation of cuprolinic blue stain. However, the relative size of cuprolinic blue (<0.5 nm) and tungstate ions used for staining of proteoglycans is probably insufficient to account for the observed differences in glycan chain thicknesses (Scott, 1992). This gives rise to the possibility that in mouse corneas chondroitin sulphate/dermatan sulphate proteoglycans aggregate laterally in the form of tetramers or polymers. Lateral aggregation of proteoglycans may be facilitated by adjacent protein cores contributing their glycosaminoglycan chains to form complementary, anti-parallel multimers through the attractive forces described previously (Fig. 6). The lateral association of several proteoglycans would confer enhanced structural integrity, and it is also possible that they may exist in heli-

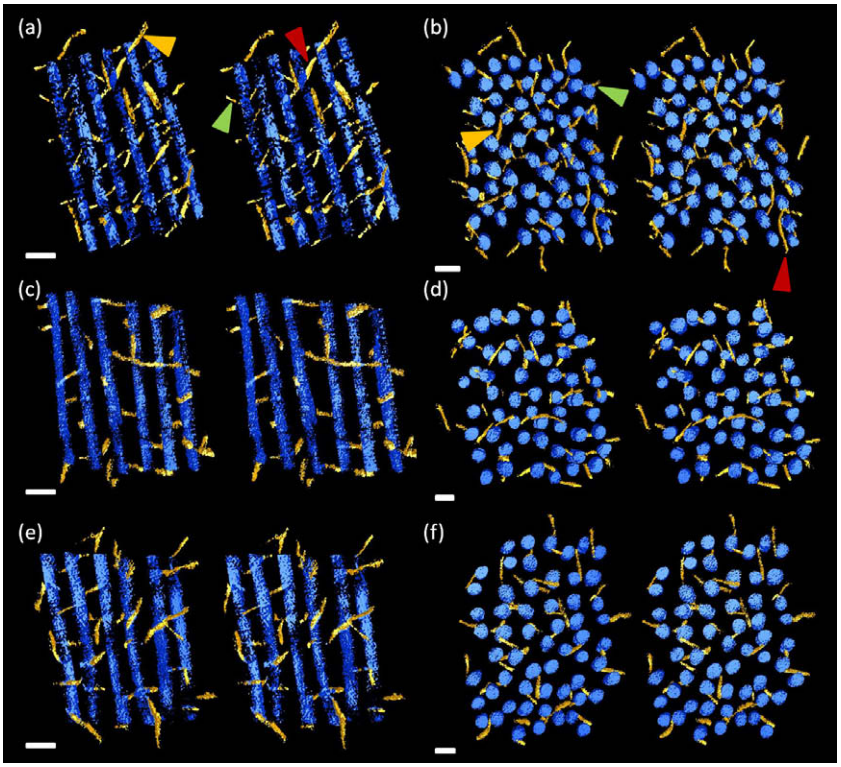


**Fig. 2.** Three-dimensional reconstructions of proteoglycan and collagen fibril organisation within the anterior (a and b), mid (c and d) and posterior (e and f) stroma of the mouse cornea. Stereo-pairs of segmented tomographic reconstructions in both (a, c and e) longitudinal and (b, d and e) transverse views. Proteoglycans are yellow in colour and collagen fibrils are coloured blue. Small proteoglycans are depicted by green arrowheads; intermediate proteoglycans are pointed out by orange arrowheads whereas the largest proteoglycans are marked by red arrowheads. Scale bar = 50 nm.

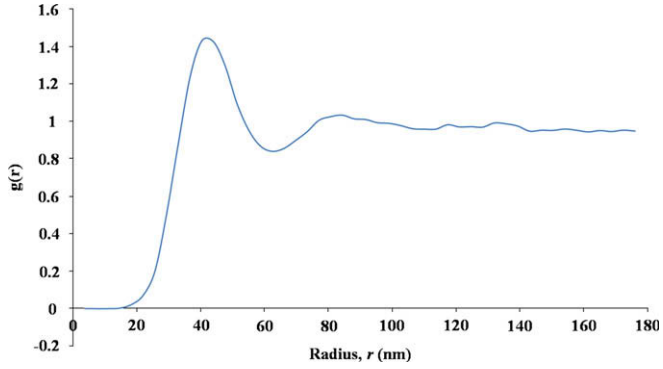


**Fig. 3.** Radial distribution function obtained from the distances of fibril centres in electron-micrographs of the anterior, mid and posterior regions of the mouse corneal stroma.

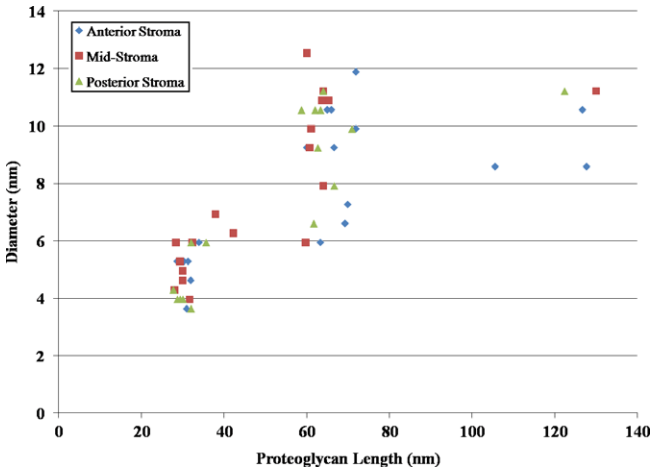


**Fig. 4.** Scatter plot of proteoglycan length versus thickness in the anterior, mid and posterior stroma of the mouse cornea. Proteoglycan measurements are restricted to the proteoglycans that display a complete structure within the reconstruction. Small proteoglycans = 5.0 ± 1.0 nm (thickness in diameter); 31.4 ± 3.5 nm (length). Intermediate proteoglycans = 9.5 ± 1.9 nm (thickness in diameter); 64.5 ± 3.9 nm (length). Largest proteoglycans = 10.4 ± 1.3 nm (thickness in diameter); 122.5 ± 9.8 nm (length).

cal conformations (Knupp et al., 2010). Multiple aggregations of this type would be able to account for the overall thickness of proteoglycans. The observed variation in proteoglycan thickness thus suggests a higher potential for self-association such that different phases of aggregation are evident. In addition, such a property could accommodate an ability of proteoglycans to readily dissociate and re-aggregate to enable fibril flexibility. This flexibility would allow for the passive movement of water and nutrients through the tissue. In addition to lateral associations of proteoglycans, it appears that end-to-end linear associations also occur, so that proteoglycans can bridge more than two adjacent fibrils. The proposed end-to-end accretion of chondroitin sulphate/dermatan sulphate may be facilitated by the dimerisation of decorin (Scott et al., 2004), the chondroitin sulphate/dermatan sulphate-containing protein core. Decorin dimerisation allows chondroitin sulphate/dermatan sulphate proteoglycans to have two opposing glycosaminoglycan extensions each of which may connect neighbouring fibrils by association of proteoglycans substituted with only a single glycosaminoglycan chain (Fig. 6). Dimerisation of decorin could potentially result in an overlapping end-to-end system extending over many fibrils, although in mouse cornea bridges of only three fibrils were most fre-

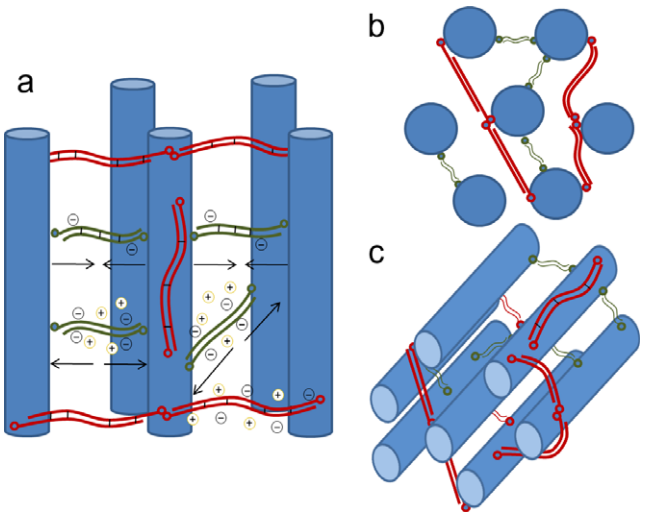


**Fig. 5.** Models of proteoglycan and collagen fibril organisation in the mouse cornea. Keratan sulphate proteoglycans are illustrated in green and chondroitin sulphate/dermatan sulphate proteoglycans are represented in red. (a) Proteoglycans are shown exerting opposite forces to control the spatial characterisation of collagen fibrils according to the charges that surround them and their thermal motion. (b and c) The non-specific order of proteoglycans is represented, reflecting the three-dimensional reconstructions. Proteoglycans interact with each other through hydrophobic and hydrogen bonding attraction with their respective glycosaminoglycan side chains, countering the mutual repulsion created by sulphate and carboxylate residues that are positioned on the repeating disaccharides. Also, negative charges may be screened by positive ions limiting repulsive forces. Chondroitin sulphate/dermatan sulphate proteoglycans have longer glycosaminoglycan chains, aggregate further and occupy a larger volume of space than the smaller keratan sulphate proteoglycans.

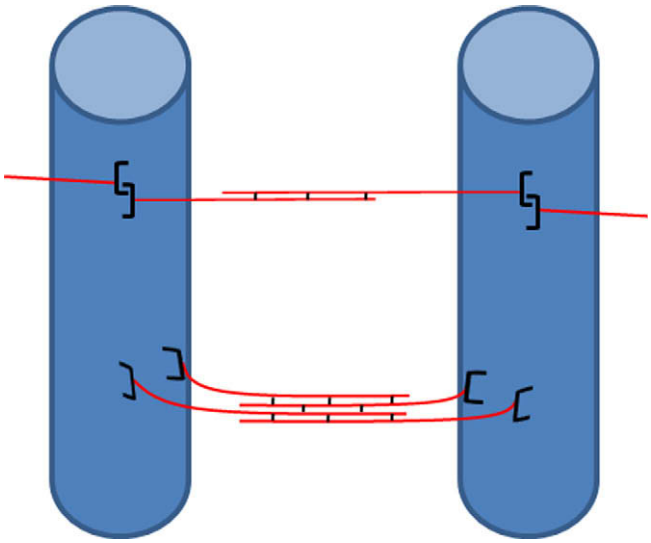

**Fig. 6.** Proposed modes of aggregation of proteoglycans. The dimerisation of decorin (black) allows the end-to-end aggregation of chondroitin sulphate/dermatan sulphate (red) proteoglycans forming an elongated proteoglycan able to attach three fibrils. Also, proteoglycans may bind to adjacent binding sites and form multiple aggregates, possibly tetramers, with proteoglycans on a neighbouring fibril.

quent. The dimerisation of decorin in solution is not universally accepted, however (Goldoni et al., 2004).

To explain the uniformity of interfibrillar distances, we propose a three-dimensional model for the mouse corneal stroma, similar to that proposed for bovine cornea (Lewis et al., 2010; Knupp et al., 2010) in which two equal but opposite forces are exerted simultaneously on the collagen fibrils (Fig. 5). As well as inhibiting the lateral accretion of collagen fibrils, proteoglycans are likely able to both contract and expand space between individual collagen fibrils in order to accurately optimise their spatial characterisation. The control of interfibrillar spacing, an important factor in the formation of corneal transparency, is governed by the glycosaminoglycan components of proteoglycans most likely according to their sulphation patterns and the consequent electrostatic forces surrounding them that permit their aggregation and dissociation. In the mouse cornea, we contend that undersulphated keratan sulphate proteoglycans form stable interfibrillar bridges that dictate interfibrillar spacing possibly thanks to their thermal motion that tends to bring their terminal ends, which are attached to adjacent fibrils, closer together. These proteoglycans would not influence stromal hydration to any great extent, conferring mechanical stability whilst also restricting osmotic swelling. Undersulphation of glycosaminoglycans results in a decrease in mutual repulsion between proteoglycans and a stable interaction between associated glycosaminoglycans arises. Keratan sulphate proteoglycans are approximately the size of the interfibrillar space between individual fibrils and so they can act as 'spacers' between fibrils.

Large chondroitin sulphate/dermatan sulphate glycosaminoglycans, where 60% is present as dermatan sulphate in mouse (Scott and Bosworth, 1990), may also control interfibrillar spacing through stabilising more than two adjacent fibrils, forming multimers and regulating the swelling pressure of the tissue through their sulphate residues. These proteoglycans, specifically in the mouse cornea, are likely to contribute to the expansion of interfibrillar spacing to a greater extent. In fact, chondroitin sulphate contains more disaccharide motifs than keratan sulphate (Plaas et al., 2001), and therefore has more potential sites for sulphation and a higher proportion of hydrophobic regions. We hypothesise that undersulphated proteoglycans, or proteoglycans with more hydrophobic regions 'pull in' fibrils to restrict, stabilise and tether interfibrillar spacing through their interactions. Conversely, highly sulphated proteoglycans capable of attracting counterions 'push out' fibrils through an influx of water because of the Donnan effect (Elliott and Hodson, 1998) (Fig. 5). Thus, proteoglycans govern the intake of water and ions from outside the tissue to control interfibrillar spacing and the overall thickness of the tissue, and are interchangeable when oxygen may not be readily available.

Collagen fibrils within the mouse cornea have a defined range of nearest neighbour separation as confirmed by the radial distribution function, although order does not appreciably extend beyond next-nearest neighbours (Fig. 3). The regularity of collagen fibril diameter, coupled with the short-range order the fibrils exhibit, is essential for mutual interference of light entering the cornea (Cox et al., 1970). Light rays impinging on collagen fibrils are scattered in all directions and if the collagen fibrils in the cornea are of equal diameter, the scattered light from each fibril will have equal intensity and wavelength. If the separation between adjacent fibrils is less than half the wavelength of the light rays, the rays scattered from all fibrils will interfere destructively in all but the forward direction so that light entering the cornea will pass through unaffected (see Hart and Farrell, 1969; Cox et al., 1970 for a detailed explanation). However, if the collagen fibrils have different diameters, as is the case in the white sclera of the eye, this is no longer the case and the scattered rays will interfere destructively, making the cornea opaque. The degree of corneal opacity depends on the degree of collagen fibril inhomogeneity, where small variations will cause only a small degree of opacity. Proteoglycans do not show, and being weak scatterers do not need to show, the same coordinated arrangement as collagen fibrils in order to guarantee transparency. The cornea, we propose, is a flexible, adaptive system in which proteoglycans adopt different, reversible conformations allowing fibrils to re-model slightly to

accommodate the passage of water and nutrients whilst preventing irreversible deformation upon impact injury and loss of transparency.

## 5. Conclusions

Proteoglycans within the mouse corneal stroma, like those in bovine cornea (Lewis et al., 2010; Knupp et al., 2010), appear to show no specific order whereas collagen fibrils maintain a regular pseudo-hexagonal arrangement. Both keratan sulphate and chondroitin sulphate/dermatan sulphate are likely to utilise their electrostatic charges and self-aggregation as a means to govern and optimise the spatial distribution of collagen fibrils. We consider that proteoglycans possibly form tetramers and in some cases extend end-to-end which allows them to adopt different conformations and connect more than two fibrils. This arrangement of proteoglycans is likely to be paramount to the transparency, flexibility and resilience of the cornea. Ongoing studies of mouse strains with genetic alterations in proteoglycans and/or glycosaminoglycans will allow us to test these conclusions more fully.

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