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Stiffness characterization of anisotropic trabecular meshwork

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ABSTRACT

Elevation of intraocular pressure has been correlated to changes in stiffness of trabecular meshwork (TM) in glaucomatous eyes although mechanical properties of the TM remain to be quantitatively determined. Data in the literature suggest that the TM cannot be considered mechanically as a uniform layer of isotropic elastic material, because the value of its Young's modulus depends on the methods of measurements and can vary up to five orders of magnitude. To this end, we proposed a new theoretical framework for mechanical analysis of the TM, in which the inner wall of Schlemm's canal and the juxtacanalicular tissue in the TM were treated as a uniform layer of isotropic elastic material, and the rest of the TM, i.e., the uveal and corneoscleral meshworks, were modeled as a uniform layer of transversely isotropic material. Using the model, we demonstrated that the large discrepancy in the apparent Young's modulus reported in the literature could be caused by the anisotropy of the meshwork that was significantly stiffer in the longitudinal direction than in the transverse direction. The theoretical framework could be used to integrate existing data of the stiffness, investigate anisotropic behaviors of the tissues, and develop new methods to measure mechanical properties of the TM.

1. Introduction

Elevated intraocular pressure (IOP) has been considered as a key risk factor for primary open-angle glaucoma (POAG) (Gordon et al., 2002; Kass et al., 2002). The disease is a leading cause of irreversible blindness due to the increase in aqueous humor (AH) outflow resistance that happens mainly at the trabecular meshwork (TM) and the inner wall of Schlemm's canal (SC) (Ethier, 2002; Johnstone and Grant, 1973; Overby et al., 2009). Although mechanisms of the increase in outflow resistance remain elusive, it is correlated to changes in the stiffness of the TM in the same eyes (Camras et al., 2012, 2014; Last et al., 2011). In general, tissue stiffness determines the extent of tissue deformation under a fixed mechanical load, which in turn can significantly alter interstitial pathways for fluid flow (Guyton et al., 1966; McGuire and Yuan, 2001; McGuire et al., 2006; Zhang et al., 2000). Thus, the flow resistance in a tissue is sensitive to changes in the tissue stiffness. Furthermore, the stiffness can affect cell behaviors through mechanotransduction (Discher et al., 2005; Overby et al., 2009), which may cause changes in AH outflow resistance as well.

Due to its importance in the clinic, different techniques have been developed to determine TM stiffness in human and animal eyes (Camras et al., 2012, 2014; Huang et al., 2015; Li et al., 2013; Raghunathan et al., 2015; Wang et al., 2016). A uniaxial stretching method has been used to measure the bulk Young's modulus of the TM isolated from human and porcine eyes (Camras et al., 2012, 2014). The average bulk modulus of the TM is observed to be 51.5 MPa for non-glaucomatous human eyes, 17.5 MPa for glaucomatous human eyes, and 2.5 MPa for normal porcine eyes. The stiffness of TM has also been measured locally in microscopic regions with the atomic force microscopy (AFM). The local Young's modulus is observed to be on the order of 100 to a few thousand pascals in mouse, rat, rabbit, porcine, and human TM (Huang et al., 2015; Morgan et al., 2014; Raghunathan et al., 2015; Wang et al., 2016; Yuan et al., 2011), which is up to five orders of magnitude smaller than the bulk modulus described above. The huge difference in the Young's moduli suggests that the bulk and local moduli reflect different structural properties of the TM. For example, the Young's moduli of collagen fibrils and cells are on the orders of gigapascal (Wenger et al., 2007) and kilopascal (Alcaraz et al., 2003; Fuhrmann et al., 2011; Overby et al., 2014; Zeng et al., 2010), respectively. At the structural level, it is harder to stretch a network of spatially aligned collagen fibers in

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the fiber direction than in the transverse direction. To integrate the stiffness data reported in the literature, we proposed a new theoretical framework for mechanical analysis of the TM, in which the tissue was characterized as an anisotropic material. Based on the model, we numerically simulated uniaxial stretching and AFM indentation of the tissue. Results from the simulation indicated that the huge discrepancy in the Young's moduli observed in the previous studies could be explained by anisotropic behaviors of the TM. The results also suggested a new approach to experimental measurement of mechanical properties of the TM.

2. Methods

2.1. Constitutive modeling of TM and surrounding tissues

TM tissue consists of three layers: uveal meshwork, corneoscleral meshwork, and juxtacanalicular tissue (JCT). For an isolated TM, its bulk stiffness depends mainly on structural properties of the meshwork. This is because the JCT and the inner wall of SC are minor components in the tissue, and they are unlikely to be significantly stiffer than corneoscleral and uveal beams. Additionally, fiber networks in corneoscleral and uveal beams are structurally anisotropic, with more collagen fibers being oriented in the longitudinal direction (Camras et al., 2012, 2014; Tamm, 2009; Tan et al., 2012), indicating that the TM is more rigid in the longitudinal direction than in the transverse direction. Physiologically, a stiffer meshwork will mechanically provide a stable support of the JCT, which is necessary to support large expansion of these tissues in the direction of aqueous humor outflow when the IOP is elevated. The expansion will increase tissue porosity, thereby reducing outflow resistance to inhibit further increase in the IOP. Data in the literature also show that the difference between tensile, bulk stiffness measured with a micro-strain analyzer (MSA) is several orders of magnitude larger than the local stiffness measured with AFM (Camras et al., 2012; Huang et al., 2015; Morgan et al., 2014; Wang et al., 2016; Yuan et al., 2011). To understand mechanisms of the discrepancy in the stiffness data, we modeled the TM as a uniform structure with two layers. The first layer consisted of the JCT and the inner wall of the SC (JIW), and was treated as an isotropic elastic material. The second layer consisted of the uveal and corneoscleral meshworks (UCM), and was treated as a transversely isotropic (TI) material with its axis of symmetry being in the longitudinal direction of the UCM.

For TI materials with linear, elastic deformation, the constitutive equation in the rectangular coordinate system can be written as:

$$\begin{bmatrix} \varepsilon_{xx} \\ \varepsilon_{yy} \\ \varepsilon_{zz} \\ 2\varepsilon_{yz} \\ 2\varepsilon_{xz} \\ 2\varepsilon_{xy} \end{bmatrix} = \begin{bmatrix} \frac{1}{E_x} & -\frac{\nu_{xy}}{E_x} & -\frac{\nu_{xz}}{E_x} & 0 & 0 & 0 \\ -\frac{\nu_{xy}}{E_x} & \frac{1}{E_x} & -\frac{\nu_{xz}}{E_x} & 0 & 0 & 0 \\ -\frac{\nu_{xz}}{E_x} & -\frac{\nu_{xz}}{E_x} & \frac{1}{E_z} & 0 & 0 & 0 \\ 0 & 0 & 0 & \frac{1}{G_{xz}} & 0 & 0 \\ 0 & 0 & 0 & 0 & \frac{1}{G_{xz}} & 0 \\ 0 & 0 & 0 & 0 & 0 & \frac{1}{G_{xy}} \end{bmatrix} \begin{bmatrix} \sigma_{xx} \\ \sigma_{yy} \\ \sigma_{zz} \\ \sigma_{yz} \\ \sigma_{xz} \\ \sigma_{xy} \end{bmatrix} \quad (1)$$

where z axis is parallel to the longitudinal direction, and y axis is parallel to the outflow direction. σ_{ij} and ε_{ij} (i and j = x, y, z) are stress and strain components, respectively. There are six constants in Eq. (1): two Young's moduli, E_x and E_z, two Poison's ratios, ν_{xy} and ν_{xz}, and two shear modulus, G_{xz} and G_{xy}; and only five of them are independent of each other. For example, the shear modulus, G_{xy}, can be calculated by

$$G_{xy} = \frac{E_x}{2(1+\nu_{xy})} \quad (2)$$

These constants can be determined experimentally through measurements of stress vs. strain relationships. The characteristic time of tissue deformation in experiments is often significantly shorter than that of water diffusion in the tissue (Camras et al., 2014; Huang et al., 2015; Long et al., 2011). Thus, both JIW and UCM were assumed to be incompressible in the study. For the UCM layer, the assumption implies that $\nu_{zx} = 1/2$,

$$\nu_{xz} = \nu_{zx}\frac{E_x}{E_z} \quad (3)$$

$$\nu_{xy} = 1 - \nu_{xz} \quad (4)$$

i.e., the number of independent constants becomes three: E_x, E_z, and G_{xz} (Loredo and Klöcker, 1997). Other tissues in the model, including JIW, sclera, and cornea, are assumed to be incompressible, isotropic, and linearly elastic.

2.2. Indentation of transversely isotropic tissues

2.2.1. Analytical solutions of spherical indentation on a half-space

The elastic indentation of a TI half-space by a rigid sphere has been solved analytically in previous studies for two situations. In the studies, the surface of the half space is either perpendicular (Dahan and Zarka, 1977; Keer and Mowry, 1979; Landau and Lifshitz, 1986), or parallel to the axis of the material symmetry (Fabrikant, 2011; Keer and Mowry, 1979; Zhupanska, 2010). In both situations, δ is related to F by

$$F = cR^{\frac{1}{2}}\delta^{\frac{3}{2}} \quad (5)$$

where c is a function of material properties (i.e., E_x, E_z and G_{xz}), and the function depends on the direction of indentation, relative to the axis of the symmetry. Expressions of the function are provided in the section of the Supplementary Information; and their derivations can be obtained from the literature (Dahan and Zarka, 1977; Fabrikant, 2011). For elastic indentation of an isotropic, incompressible half-space by a rigid sphere, the relationship between δ and F is described by the Hertz equation,

$$F = \frac{16}{9}ER^{\frac{1}{2}}\delta^{\frac{3}{2}} \quad (6)$$

where E is the Young's modulus. For other materials/tissues, Eq. (6) may also be used to characterize the relationship, except that E needs to be replaced with E_{eff}, the "effective Young's modulus,"

$$F = \frac{16}{9}E_{eff}R^{\frac{1}{2}}\delta^{\frac{3}{2}} \quad (7)$$

Comparing to Eq. (5), $E_{eff} = 9c/16$.

2.2.2. Numerical model for simulation of half-space indentation

The analytical solutions described above were used to validate numerical simulations of the indentation. The validation was performed through the simulation of a half-space indentation, using a finite element analysis (FEA) software (ANSYS Inc., PA, USA, version 14). To simplify the simulations, we first non-dimensionalized mechanical moduli, geometric dimensions, and forces by E_x, R, and R^2E_x, respectively. The dimensionless quantities were denoted in the paper, using the same symbols as those for the dimensional forms. They could be distinguished easily by checking the unit of each quantity. The half-space was modeled as a TI cubic block with a dimension of H in all three directions. The AFM probe was modeled by a rigid sphere placed at the center of the top surface of the block, and the indentation was in the downward direction. For the coordinate system shown in Fig. 1, equilibrium stress and strain distributions in the block were symmetric with respect to $x = 0$ and $z = 0$ planes. Thus, only one quarter of the block was

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