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Review

Trabecular meshwork stiffness in glaucoma

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ABSTRACT

Alterations in stiffness of the trabecular meshwork (TM) may play an important role in primary open-angle glaucoma (POAG), the second leading cause of blindness. Specifically, certain data suggest an association between elevated intraocular pressure (IOP) and increased TM stiffness; however, the underlying link between TM stiffness and IOP remains unclear and requires further study. We here first review the literature on TM stiffness measurements, encompassing various species and based on a number of measurement techniques, including direct approaches such as atomic force microscopy (AFM) and uniaxial tension tests, and indirect methods based on a beam deflection model. We also briefly review the effects of several factors that affect TM stiffness, including lysophospholipids, rho-kinase inhibitors, cytoskeletal disrupting agents, dexamethasone (DEX), transforming growth factor-β_2 (TGF-β_2), nitric oxide (NO) and cellular senescence. We then describe a method we have developed for determining TM stiffness measurement in mice using a cryosection/AFM-based approach, and present preliminary data on TM stiffness in C57BL/6J and CBA/J mouse strains. Finally, we investigate the relationship between TM stiffness and outflow facility between these two strains. The method we have developed shows promise for further direct measurements of mouse TM stiffness, which may be of value in understanding mechanistic relations between outflow facility and TM biomechanical properties.

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1. Introduction

Elevated intraocular pressure (IOP) due to increased resistance to aqueous humor outflow within the conventional outflow pathway is an important risk factor for glaucoma (Gordon et al., 2002; Grant, 1951). The principal site of outflow resistance in this pathway is the trabecular meshwork (TM), including the inner wall of Schlemm's canal (SC) (Maepea et al., 1992; Overby et al., 2009; Stamer et al., 2012). However, the exact mechanism of how aqueous outflow resistance increases in glaucoma has remained elusive. A better understanding of the pathophysiology of aqueous humor drainage through the TM would be of great benefit to the development of IOP-lowering therapies for glaucoma patients.

In this context, there are several observations which suggest that TM stiffness may be important in ocular hypertension associated with glaucoma. For example, pharmacologic modulation of TM cell actomyosin tone has a significant effect on outflow facility (see below). Further, using direct measurements of TM biomechanical properties, Last et al. (2011) reported that the compressive stiffness of TM was 20 times greater in post mortem glaucomatous human eyes compared to ostensibly healthy eyes. These findings and others reviewed below have motivated studies on the relationship between TM stiffness and ocular hypertension.

Our goal in this paper was to first review the literature on trabecular meshwork stiffness, and to then present a method and preliminary data for directly measuring TM compressive stiffness in mouse eyes. The ability to make such measurements in mice is attractive because it will hopefully allow a more mechanistic understanding of how TM stiffness and fluid flow resistance are interrelated. We begin by introducing some background material on how stiffness is defined and on factors that influence tissue stiffness.

1.1. Definition of stiffness

Stiffness is a measure of the tendency of a material to resist deformation when it is loaded, i.e. when a force is applied to it. The extent of tissue deformation can be quantified through the strain, ε; in the simplest case, strain is defined by

$$\varepsilon = \frac{tissue\ deformation}{tissue\ original\ length}$$

Similarly, the load is quantified through the stress, σ

$$\sigma = \frac{force}{tissue\ cross-sectional\ area}$$

A measure of tissue stiffness is then Young's modulus, E, defined as:

$$E = \frac{\sigma(\varepsilon)}{\varepsilon}$$

Here we explicitly note that the stress depends on the strain; in fact, for soft tissues, this dependence is usually non-linear so that Young's modulus is not a constant value, but one that varies with the strain. In this case, we can describe tissue stiffness by an effective Young's modulus, or more simply, by “the modulus”. The implication is that comparison of stiffness values from different studies is strictly only valid when the extent of tissue deformation was comparable between studies.

In addition to the magnitude of strain, the effective Young's modulus of soft tissue typically depends on a number of other factors, including how the external force is applied (i.e. direction, rate of application) and whether the tissue is in tension or compression. Importantly for any discussion of TM stiffness, it should be noted that soft tissues are much softer when they are loaded in compression vs. when they are loaded in tension.

Taking all of the above into consideration, the actual value of the effective Young's modulus should be interpreted as a general indication of tissue stiffness that may not be relevant in all situations. More realistic descriptions of tissue biomechanical behavior require more complex formulations that are beyond the scope of this article (Fung, 1993; Humphrey and Yin, 1987). Nonetheless, measured modulus values are still useful inasmuch as they can be used for relative comparisons of tissue stiffness between samples (e.g. normal vs. glaucomatous) if the testing conditions are identical between samples.

1.2. Tissue constituents contributing to TM stiffness

In general, tissue stiffness depends on both cells and extracellular matrix (ECM), and these two components interact in multiple ways in all tissues. Notably, in addition to matricellular signaling pathways and modification of the matrix by the resident cells, it is well known that cells directly sense and respond to the stiffness and topography of their underlying substrate (Discher et al., 2005; Engler et al., 2004; Georges and Janmey, 2005; Russell et al., 2008). For example, fibroblasts change their internal stiffness to try to match that of a stiffer substrate by enhancing actin polymerization and cross-linking (Solon et al., 2007). Similarly, airway smooth muscle cells increase their baseline contractile tone in response to increased substrate stiffness by upregulating their contractile protein expression (West et al., 2011).

Accordingly, we expect the stiffness of human TM to depend in a complex fashion on the resident TM cells, the ECM and the interactions between the two (Fuchshofer and Tamm, 2012; Hoare et al., 2009; Schlunck et al., 2008; Thomasy et al., 2013; Tovar-Vidales et al., 2008). It is known that TM cells are contractile (Lepple-Wienhues et al., 1991) and that elevated outflow resistance can be partly due to an increase in TM tone (Wiederholt et al., 2000). Further, TM cell contraction can direct ECM reorganization, and thus it has been hypothesized that the increased contraction state of TM cells in POAG might be associated with a

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