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Research article

Descemet membrane adhesion strength is greater in diabetics with advanced disease compared to healthy donor corneas

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ABSTRACT

Descemet membrane endothelial keratoplasty (DMEK) is an increasingly popular surgical procedure for treating ocular diseases that require a corneal transplant. Previous studies have found that tissue tearing during surgical preparation is more likely elevated in eyes from donors with a history of diabetes mellitus. To quantify these potential differences, we established an experimental technique for quantifying the force required to separate the endothelium-Descemet membrane complex (EDM) from stroma in human donor corneal tissue, and we assessed differences in adhesion strength between diabetic and non-diabetic donor corneas. Transplant suitable corneas were obtained from 23 donors 50–75 years old with an average preservation to assay time of 11.5 days. Corneas were classified from a medical records review as non-diabetic (ND, n = 9), diabetic without evidence of advanced disease (NAD, n = 8), or diabetic with evidence of advanced disease (AD, n = 10). Corneas were sectioned into 3 mm wide strips and the EDM peeled from the stroma. Using the force-extension data obtained from mechanical peel testing, EDM elastic peel tension (T_E), elastic stiffness (S_E), average delamination tension (T_D), and maximum tension (T_{MAX}) were calculated. Mean T_E, S_E, T_D, *and* T_{MAX} values for ND corneas were 0.78 ± 0.07 mN/mm, 0.37 ± 0.05 mN/mm/mm, 0.78 ± 0.08 mN/mm, and 0.94 ± 0.17 mN/mm, respectively. NAD values did not differ significantly. However, AD values for T_E (1.01 ± 0.18 mN/mm), T_D (1.09 ± 0.21 mN/mm), and T_{MAX} (1.37 ± 0.24 mN/mm) were greater than ND and NAD corneas ($P < 0.05$). S_E did not differ significantly between groups. These findings provide proof of the concept that chronic hyperglycemia from diabetes mellitus results in a phenotypically more adhesive interface between Descemet membrane and the posterior stroma in donor corneal tissue. Results of this study provide a foundation for further investigations into the impact of diabetes on the posterior cornea, eye banking, and keratoplasty.

1. Introduction

The ascendance of Descemet membrane endothelial keratoplasty (DMEK) in the treatment of corneal edema has been driven by superior visual rehabilitation and reduced graft rejection profiles compared to other cornea transplant techniques (Melles et al., 2006; Anshu et al., 2012; Hamzaoglu et al., 2015). Yet challenges presented by the tissue preparation step, in which the donor endothelium and its adherent Descemet membrane are separated from the stroma, have limited the widespread adoption of this technique (Price and Price, 2013; Terry, 2012). As with all steps of this surgery, there is a learning curve in achieving facility with DMEK tissue preparation, whether surgeons or eye bank technicians prepare the graft tissue. Even with training, DMEK graft tissue can still tear during preparation and be rendered unusable for surgery (Price and Price, 2013; Greiner et al., 2014). Thus, graft

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preparation failure is not only a costly complication, it also strains the notion that every tissue donated should be utilized in a manner that best ensures its chance to fulfill the intended purpose of restoring sight.

Our group has observed that diabetes mellitus poses a risk to successful DMEK graft tissue preparation. In a multicenter study, Greiner et al. found that diabetes increased the odds of DMEK graft preparation failure by 9-fold, as well as the time to execute successful graft preparation, compared to non-diabetic tissues (Greiner et al., 2014). This finding has since been supported by a similar independent study (Vianna et al., 2015). We hypothesize that diabetes-associated graft preparation failure is due to an increase in the adhesion strength and force required to peel the graft tissue. However, a controlled experimental investigation to confirm this association with diabetes is lacking.

In this study, we report our efforts to develop an experimental technique for measuring the adhesion strength of the endothelium-Descemet membrane complex (EDM), and to quantify potential differences in the adhesion strength of donor tissue from non-diabetics (ND), diabetics without evidence of advanced disease (NAD), and diabetics with evidence of advanced disease (AD).

2. Methods

2.1. Corneal tissue

Donor corneoscleral tissue was obtained from the following eye banks: Iowa Lions Eye Bank (ILEB; Coralville, IA), Minnesota Lions Eye Bank (St Paul, MN), Saving Sight (Kansas City, MO), Lions Eye Bank of West Central Ohio (Dayton, OH), and Lions Eye Bank of Eastern Virginia (Norfolk, VA). All tissues met transplant suitability criteria and were stored in Optisol GS (Baush & Lomb, Irvine, CA) or Life4C (Numedis, Isanti, MA) corneal storage media at 4 °C in accordance with Eye Bank Association of America standards. Eye bank medical records and specular microscopy were used to track donor age, death to preservation time, preservation to assay time, endothelial cell density (ECD), endothelial cell hexagonality and coefficient of variation, and diabetic status for each cornea.

For the purposes of this investigation, the presence or absence of diabetes mellitus was determined from a systematic review of medical records and standardized interviews with the donor family, performed by eye bank technicians trained to inquire about diabetes and diabetes complications, at the time of tissue procurement. We defined donors with diabetes as having evidence of advanced disease (AD) if the medical history review noted home insulin use and evidence of end-organ damage from diabetes such as retinopathy, chronic kidney disease, dialysis, neuropathy, peripheral vascular disease, and amputation. We defined donors with diabetes as having no evidence of advanced disease (NAD) if the medical review noted no history of home insulin use or no history of end-organ damage secondary to diabetes. We defined donors as non-diabetic (ND) if the medical history review failed to document a history of diabetes. A history of myocardial infarction was not counted as evidence of end-organ damage due to diabetes for the AD group given the high prevalence of non-diabetic coronary artery disease in our population. All diabetic donors included in this study had type II diabetes mellitus.

2.2. Sample preparation

Pre-surgical DMEK graft preparation followed the procedure described by Greiner et al. with modifications (Greiner et al., 2014). Briefly, corneas were removed from corneal storage medium (Fig. 1A) and mounted on a vacuum trephine (Barron Precision Instruments, Grand Blanc, MI). A partial thickness trephination 9.0 mm in diameter was made through the EDM into the posterior stroma. Trephined tissue was then stained with trypan blue 0.4% (Mediatech Inc., Manassas, VA) diluted in phosphate buffered saline (PBS), rinsed with PBS to remove excess stain, and cut into two rectangular strips measuring 3 mm wide and approximately 20 mm long along the temporal-nasal plane of the cornea using a custom-made tissue sectioning device (Fig. 1A). The rationale for using rectangular strips was to provide geometric uniformity between samples, improve stress homogeneity during delamination, and permit a replicate sample for each cornea. After cutting into strips, the EDM along one edge of each rectangular section was pre-peeled away from the stroma approximately 2.5 mm using non-toothed tying forceps in order to facilitate tissue attachment to the mechanical testing device. For phakic corneas, each strip of a pair of replicates was pre-peeled along a different axial region of the cornea (i.e. temporal or nasal). For pseudophakic corneas, only the nasal portion of the cornea was pre-peeled to avoid surgical incision scars in the temporal region of the cornea.

2.3. Mechanical testing

The adhesion strength of EDM to the posterior stroma was measured by devising a mechanical peel test that approximated the loading conditions the EDM experiences during graft preparation. Preliminary peel tests using a 1000 g load cell revealed that the force required to peel the EDM from the stroma was very low at approximately 1 g (i.e., 9.81 mN) and thus difficult to resolve above the background noise of the load cell. Therefore, we developed a measurement technique to measure accurately very low forces (~0.5 mN) whereby the force applied to the sample is calculated from the amount of lateral deflection induced in a bending wire placed in series with the tissue during the peel test. Our technique is based on similar methods used to measure cell-cell adhesion forces in monolayer epithelial sheets (Harris et al., 2012), investigate the flexure properties of heart valve leaflets (Gloeckner et al., 1999; Mirnajafi et al., 2006), and quantify the mechanical properties of the aortic vessel wall (Yu et al., 1993). First, a calibration curve for an 80 mm long, 0.52 mm diameter, Nitinol wire (Nitinol Devices and Components Inc., Fremont, CA) was constructed to convert lateral wire deflection to a force. This curve was produced by laterally deflecting the end of the wire a total of 5 mm in 0.5 mm increments against a rigid Teflon block positioned on an analytical balance (Mettler-Toledo Inc., Columbus, OH). For each increment of deflection, the corresponding equilibrium mass registered on the balance was recorded and multiplied by gravity to obtain the force. The data was then regressed to a line, and the slope used to convert lateral wire defections during the peel test to applied forces (Supplementary Fig. 1).

Next, each rectangular sample was clamped to a rigid testing platform and hydrated with PBS (Fig. 1B and C). Two identical 80 mm wires spaced approximately 6 mm apart were held firmly at one end by a testing fixture that was attached to the axial motor of an ElectroForce® Planar Biaxial TestBench Instrument (TA Instruments, New Castle, DE). The other ends, which extended directly over the sample, were capped with black circular markers 1 mm in diameter for image tracking purposes. One wire, referred to as the reference wire, served as a reference for position and freely translated with the linear movement of the axial motor. The other wire, referred to as the bending wire, was attached directly to the peeled end of the specimen with cyanoacrylate adhesive (Loctite, Henkel Corporation, Westlake, OH). The bending wire deflected laterally as it traveled with the reference wire in proportion to the amount of resistance it encountered from the EDM during the peel test (Supplementary Movie 1). The amount of lateral