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The inflation response of the posterior bovine sclera

Kristin M. Myers [a], Baptiste Coudrillier [a], Brad L. Boyce [b], Thao D. Nguyen [a,*]

[a] Department of Mechanical Engineering, Johns Hopkins University, Baltimore, MD 21218, USA
[b] Materials Science and Engineering Center, Sandia National Laboratories, Albuquerque, NM 87185, USA

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ABSTRACT

An *in vitro* inflation test method was developed to characterize the mechanical behavior of the bovine posterior sclera. The method used digital image correlation to provide a spatially resolved, full-field deformation map of the surface of the posterior sclera in response to controlled pressurization. A series of experiments were performed in the range of 2–6 kPa (15–45 mmHg) to characterize the load–unload displacement response at various pressure rates and the time-dependent displacement response at different applied pressures. The magnitude of the displacement was largest in the peripapillary region, mainly between the apex and the optic nerve head. Further, the results showed that bovine scleral tissue exhibited nonlinear and viscoelastic behavior characterized by a rate-dependent displacement response, hysteresis during unloading and creep. The creep rate was insensitive to the applied pressure, suggesting that the tissue can be modeled as a quasilinear viscoelastic material in the physiological pressure range of 2–6 kPa.

1. Introduction

The eye wall is the primary structural component of the eye, and it consists of a transparent cornea and opaque sclera. Both tissues serve to protect the delicate internal ocular structures from external injuries and to preserve the shape of the refractive components from changes in the intraocular pressure (IOP). The sclera is a hydrated structure containing densely stacked lamellae of type I collagen fibrils. The collagen structure has a spatially varying predominant orientation, which may be dictated by the optic nerve head (ONH) in the peripapillary region [1]. The collagen lamellae are embedded in a highly viscous proteoglycan ground substance with scleral fibroblasts sparsely dispersed in the tissue [2]. Elastin has also been found in human and animal scleral tissue in the peripapillary region surrounding the ONH [3,4]. This extracellular matrix (ECM) structure provides the sclera with the mechanical stiffness and strength needed to maintain an optimal ocular shape for vision and to mechanically support the ONH. Conditions and diseases such as myopia and glaucoma have been associated with aberrant scleral ECM structure and mechanical properties [4,5]. Glaucoma is a neurodegenerative condition caused by the progressive loss of the retinal ganglion cells (RGCs) [6] and is characterized by excessive cupping and excavation of the ONH [7]. The level of IOP is a strong risk factor for the disease, and it is hypothesized that excessive mechanical stress in the sclera generated by the level of IOP precipitates a cascade of biochemical and mechanical processes that leads to RGC apoptosis and glaucomatous damage [7–9]. Myopia, or nearsightedness, is produced by a lengthening of the eye, which moves the retina behind the focus plane. High myopia is associated with the thinning of the sclera and changes in the viscoelastic properties and ECM structure of the tissues [10–12]. Mechanical characterization of the sclera is needed to understand the development of these ocular diseases and to aid in early diagnosis and treatment.

In this paper, we present the development of a protocol for the inflation testing of the posterior sclera, which measures the spatially resolved three-dimensional (3-D) instantaneous displacement field of the tissue in response to increases in pressure. This work presents the application of this method to the characterization of the structural inflation behavior of the bovine sclera. Previous *in vitro* mechanical experiments on scleral tissue, from humans and animal models, include uniaxial tensile tests on strip specimens excised from intact sclera [11,13–18] and inflation tests on intact tissue specimens [12,19–21]. Previous *in vivo* mechanical studies include human ocular rigidity tests [22] and ocular creep tests on animal models [23]. Through uniaxial testing it was found that the mechanical properties of scleral tissue were nonlinear, heterogeneous, anisotropic and viscoelastic. Further, it was found that material properties were significantly different between normal specimens and specimens with induced glaucoma [14] and induced myopia [10,11]. Uniaxial strip tests provide an accessible method to measure and compare material behavior. However, the uniaxial loading and associated boundary conditions do not represent the complex *in vivo* mechanical loading environment. In addition, the specimen preparation process likely alters the

* Corresponding author. Tel.: +1 410 516 4538; fax: +1 410 516 7254.
E-mail address: vicky.nguyen@jhu.edu (T.D. Nguyen).

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collagen fibril structure and disrupts the natural curvature of the tissue. The former may cause the tissue to soften during the repeated loading cycle of preconditioning [13–16].

Hommer et al. [22] measured the *in vivo* ocular expansion of human eyes induced by blood pressure pulsation and reported that the eyes of glaucoma patients exhibited a higher estimated ocular stiffness than those of non-glaucoma patients. Phillips and McBrien [23] measured the change in axial length via ultrasound in cannulated and anesthetized chick and tree shrew eyes. The results showed the eyes of both animals exhibited an instantaneous elastic response to an increase in IOP from 15 to 100 mmHg. At 100 mmHg, the axial length elongated with time for chick eyes but decreased slightly with time for those of tree shrews. The difference in the *in vivo* creep behavior between the two species was attributed to the presence of active contractile myofibroblasts in the tree shrew eyes, which function to maintain the ocular shape *in vivo*. In general, *in vivo* tests incorporate the effects of mechanisms that would be difficult to replicate in an *in vitro* experiment. However, *in vivo* experiments measure the mechanical response of the entire globe, which includes the contributions of the sclera, cornea and internal ocular components. *In vitro* methods allow the mechanical behavior of the sclera to be measured separately from those of the cornea and other ocular components.

Compared to *in vitro* strip tests, inflation tests require less sample preparation, which preserves the native collagen/elastin structure and provides for a more physiological representation of the *in vivo* loading conditions. Previous *in vitro* inflation tests on human [21], porcine [20], monkey [19] and rabbit scleral tissue [12] showed that the posterior sclera deformed nonlinearly in response to pressure elevation, stiffened with age [19] and crept over long periods of time in response to a constant elevated pressure. Our method improved on the most recent inflation tests of the sclera by measuring the time-resolved and spatially resolved 3-D scleral deformation in response to controlled pressurization, which enables the characterization of the viscoelastic and regional material properties of the tissue. This paper presents a robust and repeatable *in vitro* inflation method that captures the time-dependent and spatially varying mechanical response of the sclera to increases in pressure and its application to bovine sclera. Future work will apply the method to compare the inflation response of normal and diseased human sclera to study the role of biomechanics in the development of glaucoma and myopia.

2. Methods

2.1. Material

Untreated left–right pairs of bovine eyes were harvested from animals younger than 30 months (Animal Technology, Inc., Tyler, TX). A set of specimens were used for preliminary investigations and protocol development, and a total of 10 eyes were tested using the viscoelastic testing regimen presented in Section 2.3. Specimens were shipped packed on ice, stored at 4 °C and tested within 72 h of slaughter. The specimens were not provided with culture medium to maintain the viability of the cells during storage. Therefore, some tissue degradation may have occurred. However, a previous study by Girard [14] using uniaxial tensile tests showed little difference in the viscoelastic material properties measured for fresh scleral tissue and tissue stored in PBS at 4 °C and tested within 72 h of enucleation. In addition, preliminary tests performed in our laboratory also showed a negligible difference in the inflation response of specimens tested within 72 h of slaughter. Each specimen was cleaned of orbital muscle and fat, with the ONH cut flush to the scleral surface, and kept moist by repeatedly dripping phosphate-buffered saline (PBS) during preparation.

2.2. Specimen preparation

The cleaned globes were glued to a custom-machined plastic ring from the saddle-shaped limbus to 10 mm posterior to the limbus using cyanoacrylate. The ring fixture was designed specifically to match the saddle-shape of the bovine limbus (Fig. 1A), and was described in a previous publication [24]. The locations of the nasal and temporal poles were marked on the fixture. The cornea was excised from the globe and the internal ocular components, including the choroid and the retina, were removed. To provide a rigid hold of the scleral specimen, the internal surface of the scleral rim was scored and impregnated with cyanoacrylate.

The specimen and holder were placed in a custom stainless steel pressure chamber (Fig. 1B). The design of the pressure chamber was described in a previous publication on inflation testing of bovine cornea [24]. Briefly, the pressure chamber consisted of three ports for (1) a pressure transducer (Honeywell, Sensing and Control, precision: 0.02 kPa (0.15 mm Hg)), (2) an inlet for fluid injection and (3) a release pressure valve. The inflation pressure

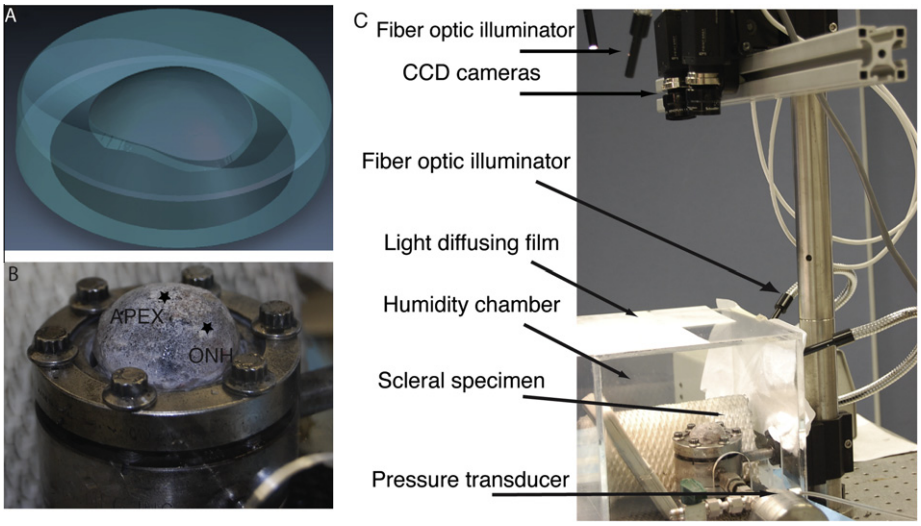

Fig. 1. (A) Saddle-shaped bovine eye inflation holder. Specimens were glued from the saddle-shaped limbus to 10 mm posterior to the limbus. For scleral inflation, the cornea was excised from the globe and the internal ocular components were removed. (B) Speckled scleral specimen in pressure chamber. (C) Experimental set-up.

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