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A simple basis for determination of the modulus and hydraulic conductivity of human ocular surface using nano-indentation

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ABSTRACT

This paper presents a simple analysis based upon Darcy's Law and indentation contact mechanics to determine the effective hydraulic conductivity and elastic modulus of fluid filled tissues. The approach is illustrated with the mechanical response of the human ocular surface using a 500 μm radius spherical tipped indenter. Indentations of various regions of the ocular surface including the corneal stroma, limbal region and sclera have been conducted. Force-control indentations were made to a maximum force, which was maintained before unloading. Measurements of the indentation response of cornea at three different loading rates were also made. Elastic like response was observed during loading, which was followed by extensive creep prior to unloading.

Statement of Significance

This manuscript attempts to provide a relatively simply model for the contact loading of fluid containing tissues and materials. It shows that the response of such materials provides a basis for determining the effective modulus and effective hydraulic conductivity (permeability) in much the same manner that hardness and modulus do for the indentation of elastic-plastic materials. Eye tissue with its anisotropic elastic and permeability properties is used to illustrate the approach.

1. Introduction

The ocular surface of the eye plays a critical role in day to day living. It guarantees a transparent cornea with a highly structured tear film for optimal optical outcomes while also providing a barrier for microbial infection. Besides being constantly exposed to "macro-mechanical" deformation by eye movements and the regular sweeping of eyelids during each blinking reflex, small scale biomechanics of the tissue itself play a critical role in tissue homeostasis [1,2]. This is particular relevant to the limbal region of the ocular surface, which lies between the sclera/conjunctiva and the corneal stroma. In this area, adult corneal epithelial stem cells reside in a highly specific niche guaranteeing the regeneration of the corneal epithelium [3]. As stem cells are sensitive to altered elastic moduli within their niche, any destruction of such a niche by disease or accident (eg heat or chemical burns), necessitates reconstruction using tailored biomaterials and tissue engineering. This situation is of critical concern for the limbal region of the eye [4–7]. These issues were the motivation to measure the elastic modulus of the ocular surface (sclera, limbal region and corneal stroma) of healthy human eyes using nano-indentation. While performing these measurements, we realized that substantial creep of the tissue occurred and that some previous approaches that only determined the nominal elastic deformation may not adequately describe the deformation of the cornea and surrounding tissues.

The tissue of the eye is well characterized and consists of highly organized primarily collagen I fibers with dermatan and keratan sulphate proteoglycans with smaller fractions of heparin sulphate between the collagen strands. In the cornea, the collagenous fibers are around 32–34 nm in diameter throughout the cornea to the limbus where fiber thickness and the extent of disorganized alignment sharply increases [8,9]. In the corneal stroma, the collagen fibers form parallel to the surface in a band with mean thickness of 550 μm that has a number of semipermeable membranes on the surface (basal membrane of the corneal epithelium) and rear of the structure (Descemets membrane) [10]. Interestingly, the

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spacing between the fibrils is 5–7% less in the central region (3–4 mm Ø) of the cornea resulting in a centrally tighter packaging of the collagenous fibers [8]. The sclera, although thicker, has a similar structure but the degree of organization of the collagen is less well ordered as in corneal stroma [8,10]. The limbal region is anatomically defined as an approximately 500 μm wide region between the conjunctiva and the cornea harboring the limbal epithelial stem cell niche in the superior 200 μm. Highly arranged radially aligned collagenous fibers from the cornea rearrange in a circumferential direction at the limbus to form an annular ring [11,12]. There is also an anisotropic depth dependence of the collagen organization of corneal tissue and associated biomechanical properties [13,14]. All these tissues are highly fluid containing with up to 80% by volume and permeable [11]. In addition, all of these are more appropriately considered orthotropic rather than isotropic structures [15].

A number of approaches have been used to measure the mechanical properties of eye tissue including indentations using air/streams (ocular response analyzer) [16,17] to those with atomic force microscopy (AFM) [18–22] and nano-indentation (NI) [23–25] as well as stress-strain response using bulge tests of the cornea or on excised tissue [26,27]. Although the NI approach was developed for testing of hard materials it has been adopted to test very soft (biological) materials [28,29]. Specific advantages of NI include: 1) ability to test local mechanical properties of extremely soft tissues (Elastic modulus down to a few kPa), 2) ability to characterize time dependent properties, 3) availability of a broad range of indenters [23], and 4) it can be done in liquids with minimal sample preparation. Generally micro-tensile or similar methods characterize the biological material as a whole while the features of interest require localized investigation [30,31]. Nano-indentation has also been successfully applied for characterization of hydrogels that have similar stiffness to many biological tissues [32,33]. Mechanical properties of the cornea to date however have been studied either by AFM [19,34] or by unconfined compression [35].

Nano-indentation (NI) analysis was developed to investigate the elastic-plastic response of materials using pointed indenters. The classic Oliver and Pharr (1992) approach, developed more than 2 decades ago and now the basis for the ISO (14557) test, enables determination of a materials hardness, which is related to the yield stress, and contact elastic modulus [36]. Shortly after this, Field and Swain (1993) used NI with small spherical tipped indenters to determine the onset of elastic-plastic behavior of materials, work hardening response [37] and phase transformation stresses [38]. Subsequently there have been numerous extensions of this approach for biological tissues including enamel, bone and cartilage [39,40,30]. The problem for all indentation tests of biological materials is the contact analysis, especially with softer biological materials, as they are characterized by relatively high fluid content (~50 to 90%) and consequently associated low (porous) solid content [41]. Analysis procedures for these materials and biological tissues are often based upon visco-elastic behavior [30,42,43]. A similar approach was adopted by Whitcomb (2011) who used a NI with a 1 mm diameter flat punch indenter [25]. Abyaneh (2013) also used NI to investigate the mechanical properties of eye tissue with a 600 μm radius indenter along with an orthotropic 3 component spring and dashpot optimization numerical analysis to fit their data [44]. Other approaches, as for articular cartilage, are to consider them as bi- or tri-phasic materials [45]. Oyen and Galli & Oyen analyzing the behavior of bone, developed an approach based upon the analysis of poro-elastic materials using an algorithm proposed by Agbezuge and Deresiewicz [46–48]. The associated method is to construct a master curve for instantaneous and infinitely slow loading responses for the normalized displacement versus normalized time of loading to extract the five parameters including the shear modulus and Poisson's ratio of the porous body, the Poisson's ratio of the fluid filled body, the permeability and the fluid pressure. Liu (2009) have also shown that it is possible to develop an equivalent visco-elastic analysis but this approach results in an even more complex inverse analysis problem [33]. Subsequently, Hu (2010) and Kalcioglu (2012) have developed a rapid indentation load-relaxation procedure that enables the shear modulus, hydraulic permeability and Poisson's ratio to be determined [29,49]. This approach also enables separation of visco-elastic from poro-elastic response of materials to be determined. The problems with all these approaches are that they are exceptionally complex inverse problems involving optimization algorithms and require a number of assumptions.

Determination of the permeability and hydraulic conductivity of eye tissue and other biological tissues is of significant clinical importance. Fluid flows through and across the surface of the cornea and plays a vital role in nutrition transport and transparency [50]. In addition, permeability is the major means for topical application of medications to the eye [51]. Measurements of permeability and hydraulic conductivity use Darcy's Law and low pressure induced flow through complete sections of excised tissue [52,53]. Measurements of localized values of hydraulic conductivity appear to be unavailable at present. Finally, the highly anisotropic structure of eye tissue is likely to influence in vivo permeability and as such the values obtained by forced flow through the thickness of the tissue with its associated semipermeable membranes may not be a genuine measure of such properties.

In this paper the emphasis is to provide a simple procedure to quantify the elastic-permeability (hydraulic conductivity) response of fluid containing tissues. As mentioned above these materials are not isotropic either mechanically or in terms of their permeability, they may have semi-permeable membranes and are likely to be graded structures. As such a rigorous analytical procedure and specific numerical model is difficult to define, hence the aim here is to develop a means to determine the *effective* contact elastic modulus as well as the *effective* permeability (hydraulic conductivity) of the tissue. It is hoped this simple approach will attract more researchers from the biological domain to engage with the biomechanics of the tissues they are involved with.

2. Materials and methods

2.1. Tissues

Corneas were obtained from the Lions Cornea Bank, Baden-Württemberg and the donors and/or relatives gave informed consent. Ethical permission for the experiments conducted was given by the ethics sub-committee of the University of Freiburg, Germany (vote no: 408/15). The corneas (details of the age and number tested are included in the results) used for the experiments were excluded from patient transplant use due to low endothelial cell counts. Before performing the experiments, corneas were equilibrated in culture medium containing 6% dextrane. The median central corneal thickness was 700 μm, which means the corneas were significantly swollen.

2.2. Nano-indentation

Nano-indentation of the eye tissue was conducted using a Bioindenter nanoindentation system (Anton Paar). A 500 μm radius ruby spherical tipped indenter was used for all tests. Indentations were made to various maximum loads between 20 and 30 μN, except for the different loading rate tests on the corneal tissue where a maximum load of 200 μN was used, with a hold period at maximum load from 60 to 180 s before unloading. Tests at the

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