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Microneedle-based minimally-invasive measurement of puncture resistance and fracture toughness of sclera

Seung Hyun Park [a], Kang Ju Lee [a], JiYong Lee [a], Jae Hyoung Yoon [a], Dong Hyun Jo [b,c], Jeong Hun Kim [b,c,d], Keonwook Kang [a], WonHyoung Ryu [a,*]

[a] Department of Mechanical Engineering, Yonsei University, 50 Yonsei-ro, Seodaemun-gu, Seoul 120-749, Republic of Korea
[b] Fight Against Angiogenesis-Related Blindness (FARB) Laboratory, Clinical Research Institute, Seoul National University Hospital, Seoul, Republic of Korea
[c] Department of Biomedical Sciences, College of Medicine, Seoul National University, Seoul, Republic of Korea
[d] Department of Ophthalmology, College of Medicine, Seoul National University, Seoul, Republic of Korea

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ABSTRACT

The sclera provides the structural support of the eye and protects the intraocular contents. Since it covers a large portion of the eye surface and has relatively high permeability for most drugs, the sclera has been used as a major pathway for drug administration. Recently, microneedle (MN) technology has shown the possibility of highly local and minimally-invasive drug delivery to the eye by MN insertion through the sclera or the suprachoroidal space. Although ocular MN needs to be inserted through the sclera, there has been no systematic study to understand the mechanical properties of the sclera, which are important to design ocular MNs. In this study, we investigated a MN-based method to measure the puncture resistance and fracture toughness of the sclera. To reflect the conditions of MN insertion into the sclera, force-displacement curves obtained from MN-insertion tests were used to estimate the puncture resistance and fracture toughness of sclera tissue. To understand the effect of the insertion conditions, dependency of the mechanical properties on insertion speeds, pre-strain of the sclera, and MN sizes were analyzed and discussed.

Statement of Significance

Measurement of mechanical property of soft biological tissue is challenging due to variations between tissue samples or lack of well-defined measurement techniques. Although non-invasive measurement techniques such as nano/micro indentation were employed to locally measure the elastic modulus of soft biological materials, mechanical properties such as puncture resistance or fracture toughness, which requires "invasive" measurement and is important for the application of "microneedles or hypodermic needles", has not been well studied. In this work, we report minimally-invasive measurement of puncture resistance and fracture toughness of sclera using a double MN insertion method. Parametric studies showed that use of MN proved to be advantageous because of minimally-invasive insertion into tissue as well as higher sensitivity to sub-tissue architecture during the measurement.

1. Introduction

Delivery of drugs to the eye for treatment of ocular diseases is challenging due to the presence of multiple tissue barriers and the continuous drainage of the eye [1,2]. Dense tissue layers such as cornea or sclera hinder the diffusion of drug molecules into the interior of the eye. In addition, administered drugs are rapidly cleared by blood flows of choroid and conjunctiva, lymphatic pathways, or tear circulation. To overcome such challenges, microneedles (MNs) have recently been studied to deliver therapeutically-useful amount of drug to the inside or the back of the eye [3]. MN technologies can provide minimally-invasive pathways to bring drug molecules into the interior or back of the eye across its protective layers such as cornea and sclera. Intra-scleral [4,5] or suprachoroidal [6,7] insertions of MNs have demonstrated effective drug delivery to the eye, and they have been regarded as promising routes for the success of ocular MN technology.

Such intra-scleral and suprachoroidal insertions of MNs require either partial insertion or full penetration of a MN through scleral tissue. The sclera comprises a large portion of the eye surface and is acellular tissue of collagen fibers embedded in glycosaminoglycan matrix [8]. The sclera mainly provides the structural support of the eye and the intraocular contents, and also protects them from injury [9]. For success of MN-based ocular drug delivery, minimally-invasive insertion or penetration of MN structures through sclera tissue is critical [10,11]. To determine the structural robustness of a MN design, it is important to understand how much force and energy are required to puncture the sclera and continue to cut the tissue [10]. There have been a limited number of studies of the elastic modulus of the sclera [12–14]. However, the scleral properties more relevant to MN insertion, such as puncture resistance or fracture toughness, have not been reported yet.

The fracture toughness of biological tissue has traditionally been measured using various technologies including tearing [15], scissor cutting [16], and blade cutting [17]. Interestingly, with recent development of robotic surgery, the fracture toughness of soft tissue has been rigorously investigated using hypodermic needles to understand needle insertion into tissue as well as its precise manipulation within the tissue [18–22]. In these works, fracture toughness was estimated by energy-based analysis using the force-displacement data of a needle insertion into soft tissue. Despite such recent development of the energy-based analysis of needle insertion, the dependency of measured values on needle insertion conditions, such as tool geometry, speeds, and pre-strain of the sample, has not been systematically investigated.

Here we aimed to measure two critical parameters of the sclera, "puncture resistance" and "fracture toughness" using a MN as an inserting tool. The two parameters bear significance to MN design for ocular drug delivery. Puncture resistance is defined as a maximum force that is required to puncture (or crack) in the sclera. Fracture toughness is defined as the amount of energy required to create a unit area of a new crack (or to continuously cut into target tissue). In particular, we studied how insertion conditions, such as insertion speeds, pre-strain of sclera samples, and the size of insertion tools, influenced the two main parameters of scleral tissue.

2. Materials and methods

2.1. Ex vivo insertion test

A tissue fixation setup was custom-fabricated to fix scleral specimens and to control the degree of their stretching (Fig. 1). Tissue samples were clamped using an aluminum plate such that the exposed region of each tissue sample was 3 mm long. One side of the fixation setup was mounted on linear bearings for free movement in a linear direction to precisely adjust the degree of tissue stretching. Since the sclera is known to have isotropic material properties [23,24], uni-directional stretching was applied to tissue samples. Tissue specimens were mounted in the setup and their pre-strains were conveniently varied from 0 to 100%. This tissue fixation setup was placed on a precision balance (10 kg capacity, Kern&Sohn GmbH, D-72336 Balingen, Germany) to monitor and record insertion forces during MN insertion tests. To measure the fracture toughness and puncture resistance of porcine scleral tissue, ex vivo tests were performed using glass MNs. Each insertion test was carried out in the following steps. First, harvested scleral tissue was suspended and stretched using the fixation setup. Then, a glass MN, which was connected to an automatic microstage, was aligned towards the center of the scleral tissue. Finally, the glass MN was lowered down at speeds of 10, 50, 100 and 250 μm/s. By recording resistance forces at the displacement increments of 1,

5, 10 and 25 μm for insertion speeds of 10, 50, 100 and 250 μm/s, respectively, a force-displacement curve was obtained for each experiment. Each test was performed either 6 or 7 times (n = 6 or 7).

2.2. Fabrication of glass MN

Glass MNs were fabricated using a micropipette puller (Flaming/Brown style micropipette puller, Sutter instrument, USA). After a glass capillary tube was pulled to have a desired diameter and shape, the tip of the micropipette was forged to close the open end. In this study, we fabricated glass MNs that had different tip diameters by three steps of pre-programmed pulling. First, a glass capillary (1.0 mm O.D., 0.75 mm I.D.) was mounted in the micropipette puller and pulled at three steps of increasing pulling forces and speeds. Afterwards, the tip diameters of the glass MNs were finely adjusted with a microforge (MF-830, Narishige group, Japan). Using the micropipette puller, parameters such as heating temperature, cooling times, pulling forces and speeds were adjusted to control the shapes of glass MNs. The tip diameters of MNs were precisely shaped by changing the distance between the heating filament of the microforge and MN tips (Fig. 2). The gaps were 40, 20, and 10 μm for fabrication of the tip diameters of 9, 25, and 50 μm, respectively.

2.3. Tissue specimen preparation

In order to perform ex vivo insertion tests for the measurement of mechanical properties of ocular tissue, fresh porcine eye globes were purchased from a local market and prepared on the same day. The prepared samples were stored in a refrigerator and used within 2 days. Redundant tissue was excised from scleral surfaces and then the cornea of the eye globe was cut out along the limbus with surgical scissors. Subsequently, the eye globe was opened and the vitreous humor and retinal tissue was gently removed from the vitreous side of the sclera using a surgical tweezer. All of the scleral tissue were prepared in a uniform size (width 7 mm, length 5 mm) for insertion tests. To reduce measurement errors from thickness variation, the scleral samples were prepared at a distance of 5 mm from the limbus (Fig. 1C). This location was selected since it was the thinnest part of the sclera where subconjunctival injection was generally applied. The thickness of the sclera samples was 364.3 ± 13.8 μm on average.

After insertion test, scleral tissue samples were imaged using high resolution optical coherence tomography (OCT) to visualize cracks formed by MN insertion. All the tissue samples were pre-strained at 50% before image acquisition, and then immersed in a water chamber to minimize the effect of specular reflection from the surface.

2.4. Double insertion method for measurement of fracture toughness and puncture resistance

2.4.1. Energy balance equations

When a glass MN penetrates scleral tissue during insertion tests, a crack forms in the tissue and the crack propagates under continuous loading. A force-displacement (F vs. x) curve of ex vivo insertion test can be utilized to estimate the fracture toughness of scleral tissues (Fig. 3A). In the force-displacement curve, the 1st peak indicates the onset of MN penetration (crack formation) and the subsequent gradual increase in the force corresponds to crack propagation, elastic deformation energy, and frictional resistance during further MN insertion into the tissue. In order to measure fracture toughness after the 1st peak of MN puncture, an energy balance equation can be established like the following [19,20]: