# AN EXPERIMENTAL INVESTIGATION ON BENDING RESPONSE OF SCLERA TISSUE UNDER ELECTRICAL STIMULATION

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## INTRODUCTION

The sclera is the shell surrounding the eyeball attached to the cornea. It is a dense connective tissue constituting more than 80% of the eye's wall. The tough nature of the sclera protects the eye from serious damages as well as also providing a solid support for the extraocular muscles. From the microstructure point of view, the main compositions of the sclera include collagen, proteoglycans (PG) and elastin. PGs are polysaccharides units which are attached to glycosaminoglycans (GAGs) side chains. The GAGs play a key role in determining hydration, elasticity and resiliency of the sclera tissue. The main chemical compositions in the GAGs structure are carboxyl (i.e., $COO^-$) and sulfate (i.e., $SO_3^-$) groups. Within the physiological pH range (pH≅7.0) the GAGs are negatively charged, and the sclera tissue is considered as a natural polyelectrolyte hydrogel [1]. When an electroactive hydrogel is placed in a salt solution (e.g., NaCl) between two electrodes, upon the application of electrical voltage to the electrodes, the hydrogel bends towards one of the electrodes. The direction of the bending depends on the sign of the electrical charge inside the samples; a negatively charged gel bends to the cathode while a positively charged hydrogel bends to the anode. The sclera is a negatively charged tissue, so it is expected to behave like an electroactive polyanionic hydrogel, i.e. show a mechanical response when subjected to external electrical stimulations. The objective of the present work was to perform experiments and test the above hypothesis.

The electrical stimulation leads to water transportation due to migration of the counterions of the fixed charges inside the hydrogel (i.e., electroosmosis) [2]. The mechanism proposed for this electroactive bending response is the transient alteration in concentration of mobile ions (e.g., $Na^+$ and $Cl^-$) at the boundaries of the hydrogel and surrounding solution leading to local swelling/shrinking at the gel-solution interfaces. Aside from the content of the fixed charge, mechanical properties of the hydrogel, strength of the electrical field, the pH of the surrounding medium are other important contributing factors in electromechanical bending response of a polyelectrolyte hydrogel.

In the present study, we conducted experimental measurements to demonstrate the electro-responsiveness of sclera and investigate the effects of magnitude of the electrical filed on bending response of the tissue.

## METHODS

Fresh porcine eyes were collected from slaughterhouse (n = 18). After cleaning the globes by removing the fat and muscle, the globes were dissected, and sclera strips with dimensions of 2.5 mm × 20 mm were excised from the posterior of the eye globe in superior-inferior direction with 2 mm distance from the optic nerve head. The sclera strips were dried in desiccator for 24 hours. After measuring the weight of the dried samples, the strips were placed in 0.15M NaCl solution. The weight of the samples was monitored in time and their hydration H was calculated as

$$H = \frac{W_s - W_d}{W_d}$$

where $W_s$ is the wet weight of samples and $W_d$ is their dry weight. After the samples reached to hydration of 2 mg water / mg dry weight, they were mounted in experimental setup. For conducting the experiments, we designed a container with two carbon electrodes 5 cm apart along with clamps for fixing the tissue from one side inside the container. We used a DC power supply for applying the electric voltage. In addition, we used a digital microscope to track the electromechanical bending response of the sample inside the container filled with NaCl solution (0.15M concentration). For this purpose, we developed a program in Python in order to capture the movement and calculate the bending angle of the sample in real time. The main components of the experimental setup are shown in Figure 1. The bending angle of the samples was defined as deviation of their tip from the initial position as shown in Figure 2.

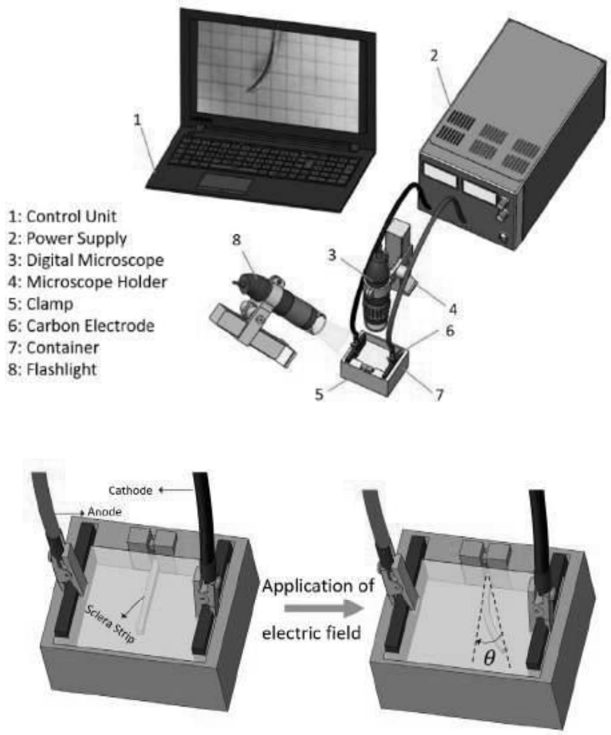


**Figure 2: Illustration of different components of the experimental setup. Sclera strips were mounted in the setup immersed in NaCl solution and their deformation was captured by a digital camera. The bending angle θ quantifies the amount of their bending under electrical stimulations.**

It is expected that the bending angle of the samples to depend on the pH of the solution since any change in the solution pH could change the amount of fixed charges inside the tissue. Upon applying the voltage, pH waves will be generated because of electrolysis of water. We used a universal pH indicator to monitor propagation of pH waves inside the container under different voltages.

## RESULTS

It was seen that with increasing the voltage, the pH waves propagate faster into the solution. For example, it took more than 2 minutes for pH waves propagating from the electrodes to reach the center of the container when an electrical voltage 15 V was used. It is also known that speed of pH wave propagation is proportional to the magnitude of the applied voltage. Since 15 V was the maximum voltage that we applied in the present work, we limited the duration of our experiments to 60 seconds in order to limit the pH variation inside the solution surrounding the samples.

The bending deformation of a typical sample under electric voltage is shown in Figure 1. Furthermore, the electromechanical bending response of scleral strips at 5 V, 10 V and 15 V of electrical stimulations was plotted as a function of time in Figure 2. It is seen that with increasing the voltage and time, the bending angle increases. The rate of scleral bending was fast in the beginning and it slowed down with increasing time.

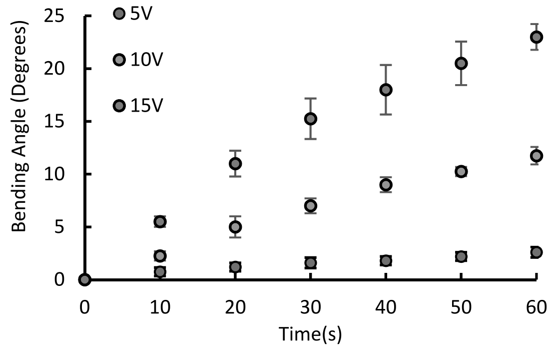


**Figure 2: The bending angle of scleral strips subjected to 5 V, 10 V and 15 V as a function of time.**

## DISCUSSION

The scleral samples showed mechanical bending response to the external electric voltage. We observed that samples subjected to 5V, 10V and 15V for 60 seconds reached to a maximum bending angle of nearly 3°, 10° and 23°, respectively. Thus, it is concluded that the deformation of sclera was significantly dependent on the magnitude of the applied external electrical field. This observation is in agreement with previous studies on the electroactive bending behavior of electroactive hydrogels [3]. This dependence on the strength of the electrical field is because increasing the voltage leads to increasing the speed of movement of the ions inside the solution. Subsequently, differential osmotic pressure at the boundaries of the samples and solution is created more quickly, which in turn increases the mechanical bending response of the tissue. The present experiments had some limitations. For example, the electrolysis of water and creation of pH gradient in the solution may have affected the electromechanical response of the tissue. This is because the pH waves alter the net electrical charges inside the tissue. In the present work, the strength of electrical field and time of electrical stimulation were limited to minimize the possible effects of pH gradient on electromechanical bending response of the samples. The present work characterized the electromechanical response of the sclera and its findings revealed some of the biochemical as well of biomechanical properties of this ocular tissue. We have also developed numerical models and tuned them against the experimental data [4]. We are currently working on applying the experimental framework of the present study to other biological tissue containing fixed electrical charges in order to increase our understanding of their electromechanical properties.

## ACKNOWLEDGEMENTS

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