Tensile properties of porcine retina

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***Abstract*—The mechanical properties of the retina has not received much attention, probably due to the fact that retina is very fragile and that its main function is not related to mechanical support. In this study, we provide a protocol to isolate the retina and perform tensile testing on porcine retinal tissues. Moreover, we present our preliminary results. These show a short linear region followed by a relatively wide non-linear region characterized by peaks. Additional tests will be carried out to confirm these preliminary findings.**

***Keywords*—Retina, tensile test, mechanical parameters, ophthalmology.**

I. INTRODUCTION

THE retina, lining the posterior wall of the eye, is a thin neurosensory membrane responsible for absorption of the light and transmission of this signal to the brain through the optic nerve. It is composed of multiple layers of different cells including retinal epithelial cells (RPE), photoreceptors, glial cells and neurons (Fig.1). These cells are supplied either by the central retinal artery and its branches which perfuse the anterior retina or by the choroid that provides nutrients and oxygen to the outer retina through Bruch’s membrane (BM).

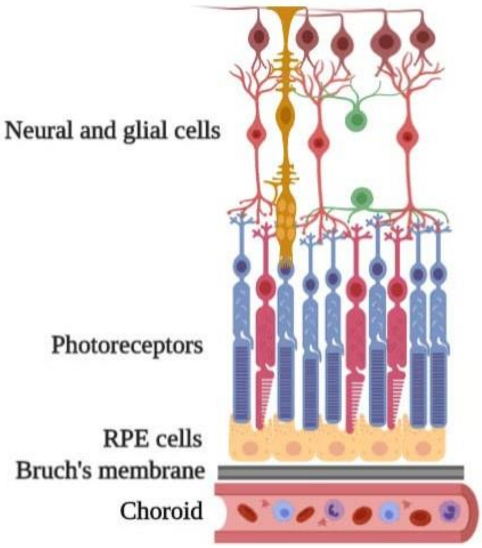


Fig. 1: Schematic illustrating the cellular composition of the retina

The mechanical properties of the retinal tissue are scarcely investigated probably because of its extreme structural fragility [1], [2], [3]. Hence, retina results to be difficult to be isolated and manipulated.

Although the retina does not play a key role in mechanical support, the knowledge of its mechanical response is important for a variety of reasons. For instance, it helps completely understand the retinal physiology. In addition, it allows to build both *in silico* and *in vitro* models used for investigating retinal physiology and pathologies such as age-related macular degeneration (AMD), glaucoma, retinitis pigmentosa, diabetic retinopathy [4], [5]. In high-income countries, these diseases account for a large proportion of blind or visually impaired people [6]. Moreover, they are difficult to treat, mostly because topical administration presents limited access to the retina and systemic administration requires high doses with consequent undesired effects. Intravitreal injections represent an alternative but they are invasive, expensive, and unable to restore tissue functionality [6]. Knowing retinal biomechanical properties is also fundamental to develop retinal functional tissue engineered prostheses that can be implanted in patients suffering from one of the diseases previously mentioned. Another reason to examine mechanical properties of retinal tissue is to understand and to prevent the risks of detachment during surgical procedures (i.e. vitrectomy). Indeed, during vitrectomy, which consists in removal and consequent replacement of the vitreous, retinal tear can be caused with subsequent cell loss resulting in retinal detachment [1].

Here, we present the preliminary results of tensile tests performed on pig retinal tissues.

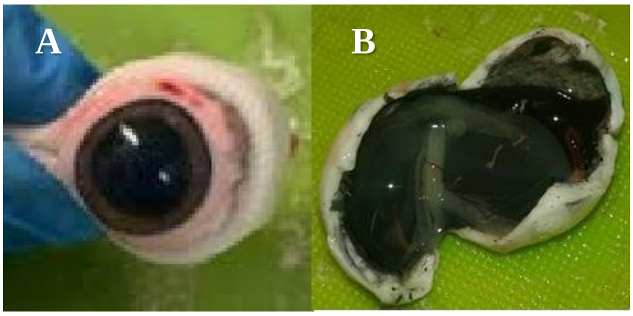
II. MATERIALS AND METHODS

A. Sample collection

Pig eyes were collected from a local abattoir (Fumagalli Industria Alimentare, Tavernerio (CO), Italy), within 1 hour post mortem. Pigs were nine months old.

B. Sample preparation

The eyeballs were cleaned from adipose tissue and conjunctiva (Fig. 2A). Using a scalpel to make a circular cut on the sclera at the cornea level, we carefully removed, in sequence, cornea, iris, crystalline lens, and vitreous (Fig. 2 B). Then the retina was cautiously detached from the back wall of the posterior chamber (Fig. 2C), and preserved in PBS solution (P4417, SIGMA) at 4 °C until testing. Before testing, 11x5 mm retinal samples were obtained.



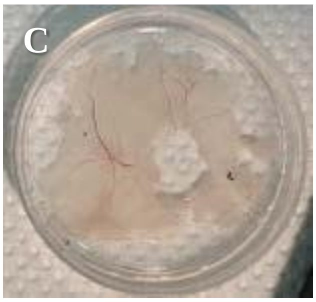


Fig. 2: **(A)** Clean eyeball after adipose tissue and conjunctiva removal. **(B)** Isolation and detachment of retina from the back of the eye. **(C)** Detached retina preserved in PBS.

C. *Experimental apparatus and protocol*

For the mechanical measurements, retinal samples were clamped between the grips of an electromechanical testing machine (TC3F, EBERS), equipped with a 1 lb load cell (Fig. 3). Grips were made of knurled stainless steel and clamping was obtained manually by screws. Silicone sheets were interposed between clamps and retina to preserve the sample integrity. After five preconditioning cycles, consisting in stretching the samples up to 1 mm and back to zero at a strain rate of 0.88 %/s, samples were pulled until failure at a velocity of 0.88 %/s at room temperature.

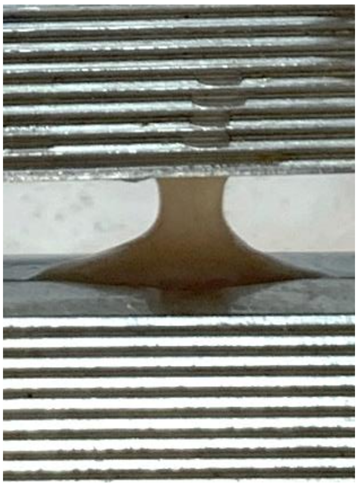


Fig. 3: Porcine retina clamped between the grips of the tensile testing machine

D. *Definition of Biomechanical Terms*

From the force (N) – elongation (mm) data, we calculated the stress (MPa) as the ratio of the applied force to the original cross section area (mm^2), and the strain as the change of length divided by the original length. The original cross section area was calculated as the multiplication of the initial thickness by the initial width, both evaluated through the ImageJ software (National Institutes of Health, USA). The gap between the clamps was considered as the original length. The elastic modulus (E) was extracted from the stress-strain plots as the linear portion of the curve before the change of the slope. The yield stress (Y) was defined as the point of change of slope in the first linear elastic region of the stress-strain curve. The failure stress was set to the maximum stress value.

E. *Statistical analysis*

Results of E, Y and failure stress are presented as average ± standard deviation (SD).

III. RESULTS

A. *Stress-Strain Measurements*

A typical stress-strain curve of retinal samples under tensile tests is shown in Fig. 4. The curve exhibited a narrow initial linear region followed by a wide region of plastic behavior presenting several peaks probably due to micro-ruptures in the tissue.

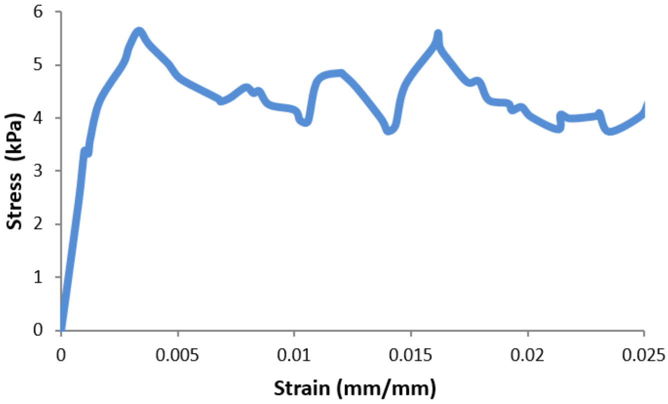


Fig. 4: Tensile stress versus strain curve of retinal specimens at room temperature

A close-up view of the first portion of the curve displays an initial elastic region followed by yielding before rupture.

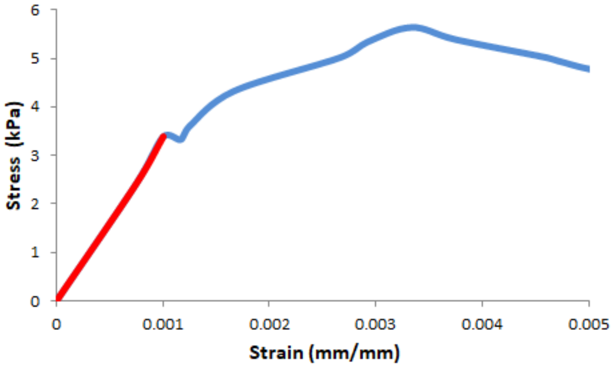


Fig. 5: Zoom in of the stress-strain relationship shown in Fig. 4. The red line represents the initial elastic region before plastic deformation.

For the stress-strain measurements of retinal tissue, five samples (n=5) were tested and their resulting curves were analyzed. The elastic modulus E at room temperature was on average 2.87 ± 1.29 MPa with values ranging from 1,65 MPa to 5 MPa. The Y and the failure stress resulted to be 3.67 ± 2 kPa and 7.428 ± 3.62 kPa respectively. Table I outlines the average values and the SDs of the biomechanical parameters taken into account, namely E, Y, and failure stress.

TABLE I
BIOMECHANICAL PARAMETERS

Parameter	*Average value*	*Standard deviation (SD)*
Elastic modulus (E) (MPa)	2.87	1.29
Yield stress (Y) (kPa)	3.67	2
Failure stress (kPa)	7.428	3.62

Table I shows the average values and the SDs of the biomechanical parameters analysed in this study

IV. DISCUSSION AND CONCLUSION

Considering the initial tests (n=5) that we performed, we can hypothesize that the biomechanical behaviour of the retina is characterized by a short linear region of elastic deformation and a relatively wide non-linear region of plastic deformation. Nevertheless, these results are still preliminary. Hence, currently we are carrying out additional tests that are necessary to obtain more significant data and to compare our findings with those of the previous studies [1], [3].

ACKNOWLEDGEMENT

The authors would like to thank Dr. Fumagalli and his Fumagalli Industria Alimentari located in Como for kindly providing the porcine eyes.

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