

Novel 3D *in vitro* retina model to investigate mechanisms involved in age-related macular degeneration

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Abstract

In the present study, we present a strategy to develop a 3R-compliant *in vitro* retina model to study age-related macular degeneration pathways.

Introduction

Age-related macular degeneration (AMD) is one of the leading causes of irreversible vision loss worldwide, affecting globally 170 million people.¹ AMD consists in a progressive degeneration of the retinal pigment epithelium (RPE) and the underlying Bruch's membrane (BrM). Current treatments are unable to reverse vision damage. This is related to incomplete knowledge of the basic pathological mechanisms involved. So far, animal models have been employed to investigate AMD pathways. However, high-scale *in vivo* methods are complex to establish. In addition, the 3R principle has urged to use alternative approaches. Hence, in the present study, we aimed to develop a suitable 3R-compliant three-dimensional (3D) *in vitro* model, including a BrM model and the RPE layer. To build an *in vitro* model that mimics the native tissue, we investigated the biomechanics of native porcine retina. In line with the 3R principle, pig eyes were collected from a local abattoir applying the reuse of animal, thus reducing the number of animals used for research purposes.

The *in vitro* model will be crucial to study and acquire greater insight into the pathological mechanisms involved and to develop and test new therapies, which cure AMD.

Materials and Methods

BrM model was fabricated with the EF300 electrospinning system (SKE

research equipment, Leonardino s.r.l.) using a 15% (wt/v) polymer solution, composed of polycaprolactone (PCL, Sigma) and Bombyx mori Silk Fibroin (SF) (weight ratio 95:5 PCL/SF).

BrM model was characterized in terms of morphology, mechanical properties, permeability, and biocompatibility. Morphological analyses were performed using scanning electron microscopy (SEM, Stereoscan 360, Cambridge Instruments) at 10 kV and ImageJ software (National Institute of Health). A mechanical testing machine (Synergie 200, MTS Systems) equipped with a 100 N loading cell was used for tensile test. Samples were first pre-conditioned and then pulled to failure at 0.1 mm/s. The same protocol was applied to hydrated samples, *i.e.* samples submerged in saline solution for 1 hour. Stiffness was calculated by measuring the slope of the initial linear region of the resulting stress-strain curve. To estimate permeability, a custom-made apparatus was employed. Constant hydrostatic pressures were applied to the sample and the flow rate was measured to calculate Darcy's permeability and then hydraulic conductivity. To investigate the *in vitro* biocompatibility of the BrM model, a direct contact cytotoxicity test was carried out for 5 days using SEM. 4×10^5 ARPE-19 cells (ATCC) were seeded on the BrM electrospun model. For retinal mechanical measurements, 9-month-old pig eyes were collected within 1 hour post-mortem. Retinal samples were isolated and pulled until failure at 0.1 mm/s by using an electromechanical testing machine (Bose EnduraTEC ELF 3200, Eden Prairie) equipped with a 22 N load cell. The elastic modulus (E) was extrapolated from the stress-strain curves as the linear portion of the curve before the change of the slope.

Results are presented as mean \pm Standard Deviation (SD). Differences were considered statistically significant if the *p*-value was less than 0.05.

Results

The fabricated BrM model presented a randomly oriented fibrous network with an average fibre diameter of 1217 ± 101 nm and a thickness of 44 μ m. The mechanical properties of the BrM model were reflected by a typical tensile stress-strain curve. No

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statistically significant difference was found between dry and hydrated test conditions. The average values of stiffness and hydraulic conductivity of the BrM model were in agreement with those estimated for native BrM (Table 1).^{2,3} Finally, BrM model proved to be biocompatible, as cells well adhered and spread on its surface (Figure 1). Based on BrM biocompatibility, RPE layer will next be bioprinted on the BrM

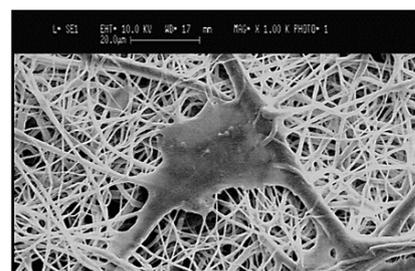


Figure 1. SEM image of an ARPE-19 cell on the BrM model.

Table 1. Stiffness and hydraulic conductivity of the BrM model and native BrM.

Parameter	Native BrM	BrM model
Stiffness [MPa]	7-14 [2]	13 \pm 3.6
Hydraulic conductivity [m/(Pas)]	From 20 to 100×10^{-10} [3]	20×10^{-10}

model. To develop a suitable bioink, a material with appropriate mechanical properties has to be selected. However, retinal mechanical properties have been poorly characterized. Therefore, we carried out tensile tests on porcine retina. The resulting stress-strain curve of retinal samples under tensile tests is shown in Figure 2. The curve exhibited a narrow initial linear region followed by a wide region of plastic behavior

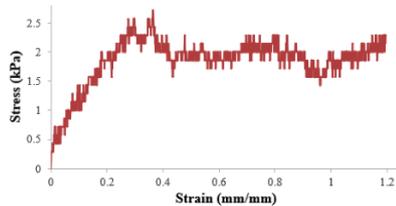


Figure 2. Stress-strain plot of porcine retina under tensile test.

presenting several peaks probably due to micro-ruptures in the tissue. The average value of E was 13.4 ± 6.7 kPa. This outcome will help us to build an *in vitro* retina model that better replicates the native tissue.

Discussion and Conclusions

In this study, novel BrM-mimetic substrates were fabricated as part of an *in vitro* retina model. The substrates displayed structural and mechanical similarity to physiological BrM. Moreover, we proved that the BrM model can be used as a substrate for RPE layer. To better reproduce RPE layer, we characterized retinal biomechanics with a tensile test. Thanks to this outcome, we will develop a suitable bioink for the 3D Bioprinting of RPE on the BrM model, thus obtaining a 3D *in vitro* retina model. The model will be used to study

AMD progression and develop new effective therapies, thus reducing the number of animal experiments.

References

1. Wong WL, Su X, Li X, et al. Global prevalence of age-related macular degeneration and disease burden projection for 2020 and 2040: a systematic review and meta-analysis. *Lancet Glob Health* 2014;2:e106-16.
2. Ugarte M, Hussain AA, Marshall J. An experimental study of the elastic properties of the human Bruch's membrane-choroid complex: relevance to ageing. *Br J Ophthalmol* 2006;90:621-6.
3. Moore DJ, Clover GM. The Effect of Age on the Macromolecular Permeability of Human Bruch's Membrane. *Invest. Ophthalmol. Vis Sci* 2001;42:2970-5.