Toward Bruch's Membrane Mimicry: Electrospun PLGA and Gelatin Substrates for ARPE-19 Barrier

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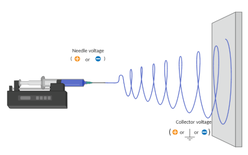
Introduction

Engineering a functional **Bruch's membrane (BrM)** analogue able to support a stable retinal pigment epithelium (RPE) monolayer is essential for developing **physiologically** relevant outer **retinal models**.[1] Among candidate biomaterials, **PLGA** and **gelatin** represent promising BrM substitutes.[2]
This study evaluated **electrospun PLGA** and **gelatin-GPTMS membranes** as BrM-mimicking substrates for **ARPE-19 cell** culture, using **Transwell®** supports as reference controls.[3]

Material and Method

Membrane fabrication

- **Gelatin** was **crosslinked** with **GPTMS**
- Gelatin-GPTMS and PLGA membranes were fabricated by **electrospinning**
- Processing parameters were optimized to obtain **uniform nanofibrous membranes**



Solution electrospinning

Structural characterization

- Membrane morphology was examined by **scanning electron microscopy (SEM)**
- **Fiber diameter** distribution was analyzed using **GIFT/ImageJ**



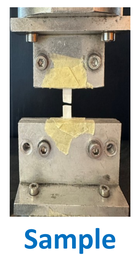
Scanning electron microscopy (SEM)

Functional and mechanical characterization

- **Diffusivity** and **permeability** were evaluated using custom diffusion and hydrostatic-pressure systems
- **Mechanical properties** were assessed under **dry** and **wet** conditions by uniaxial tensile testing



Tensile test

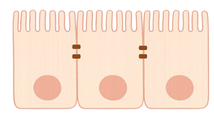


Sample failure

Biological evaluation

- **ARPE-19 cells** were cultured on the membranes for **28 days**
- **Cytocompatibility** was assessed by **MTT** and **LDH assays**
- **Barrier maturation** was evaluated by **TER** and FITC-dextran **permeability**
- **Tight junction** organization was analysed by **ZO-1 immunofluorescence**

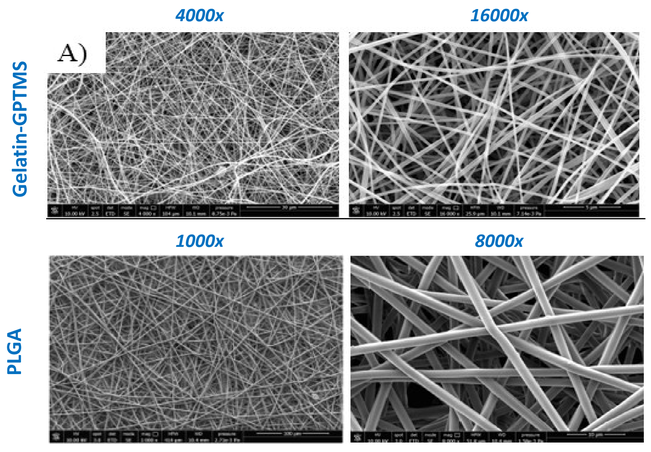
ARPE-19 cells



Results

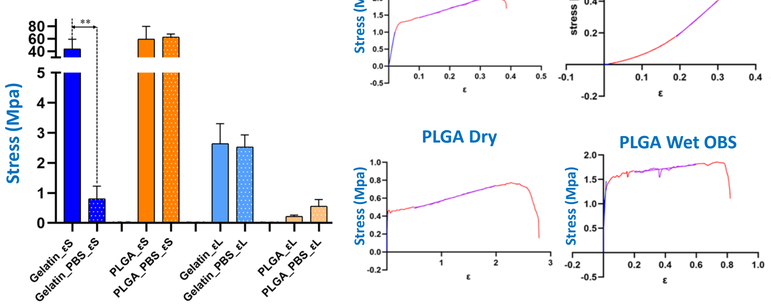
Morphology

- **Homogeneous** nanofibrous **morphology** in both membranes
- **Gelatin-GPTMS**: random, straight, aggregate-free fibers
- **PLGA**: larger fiber diameter



Mechanical properties

- **Gelatin-GPTMS**: reduced stiffness and strength in wet conditions
- **PLGA**: higher mechanical stability



Transport properties

- **Both membranes**: diffusivity comparable to native BrM
- **Gelatin-GPTMS**: highest diffusivity and higher permeability
- **PLGA**: permeability closer to native BrM

Table 1. Permeability Estimation

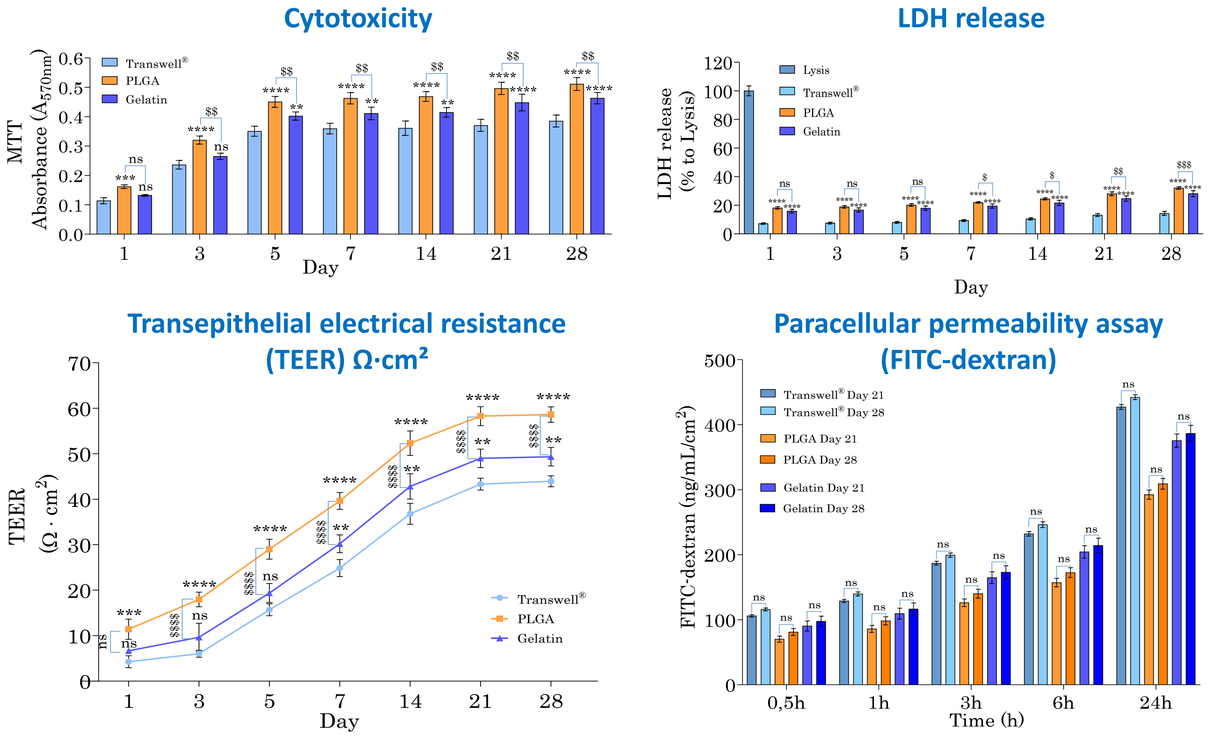
Membrane	Permeability, K (m^2)	
	DI water	PBS
Bruch's Membrane	(2±0.2)*10-17	(2 ±0.2)*10-17
Gelatin-GPTMS	(1.07±0.1)*10^{-14}	(1.06±0.37)*10^{-14}
PLGA	(7.7 ±0.1)*10^{-18}	(6.44 ±2)*10^{-18}

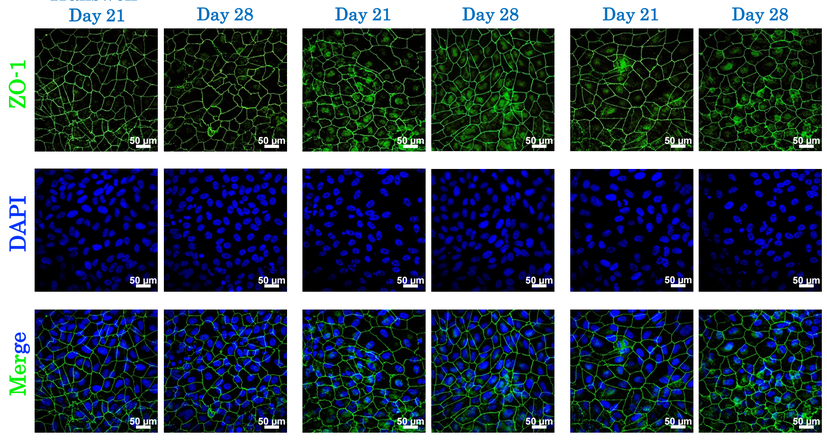
Table 2. Diffusivity estimation

Membrane	Diffusivity, D (cm^2/s)	
	Methylene Blue (319,85 g/mol)	L-DOPA (197,18 g/mol)
Bruch's Membrane	10^{-6}	10^{-6}
Gelatin-GPTMS	(2.8±0.8)*10^{-5}	(1.0±0.4)*10^{-5}
PLGA	(2.0±0.3)*10^{-7}	(5.0±0.6)*10^{-7}

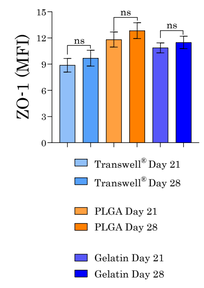
Cell response and barrier maturation

- Better **ARPE-19 viability** and **monolayer formation** than Transwell®
- **TEER increased** until **day 21**
- **PLGA** showed the **highest TER** and **lowest FITC-dextran flux**
- **ZO-1** was more defined on **PLGA**





ZO-1 mean fluorescence intensity (MFI)



Conclusion

Electrospun **PLGA** provided the most favourable overall platform, combining biomimetic transport properties, mechanical stability, cytocompatibility, and enhanced **ARPE-19 barrier maturation**. These findings support its use as a **BrM analogue** for outer retinal models and retinal drug permeability studies.

Reference:
1. Dunn, K. C., Aotaki-Keen, A. E., Putkey, F. R., & Hjelmeland, L. M. (1996). ARPE-19, a human retinal pigment epithelial cell line with differentiated properties. Experimental Eye Research, 62(2), 155–169. https://doi.org/10.1006/exer.1996.0020
2. Thomson, H. A. J., Treharne, A. J., Walker, P., Grossel, M. C., & Lotery, A. J. (2011). Optimisation of polymer scaffolds for retinal pigment epithelium (RPE) cell transplantation. British Journal of Ophthalmology, 95(4), 563–568. https://doi.org/10.1136/bjo.2009.166728
3. Zhang, F., Liu, L., Zhang, H., & Liu, Z. L. (2020). Effect of platelet-activating factor on barrier function of ARPE-19 cells. Drug Design, Development and Therapy, 14, 4205–4214. https://doi.org/10.2147/DDDT.S251941