A new poly(carbonate)urethane/gelatin electrospun membrane for physiological barriers in in vitro models

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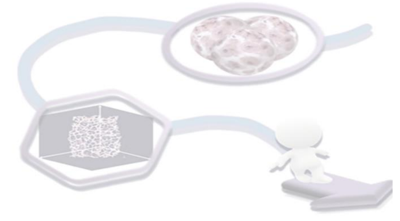

ABSTRACT

In the human body, physiological barriers allow the separation between different compartments of the body or with the outer environment, acting as the first level of defense against microorganism, toxins and allergens. Moreover, these barriers have a fundamental role in the control of absorption of substances and the maintenance of the homeostasis of the different body compartments. For these reasons, the study of biological barriers is crucial not only for a better understanding of their physiology and pathology, but also in drug testing and toxicology studies. Barrier-forming cells are often cultured in fluidic systems (bioreactors) able to apply dynamic conditions [1]. The main actor of these systems is the porous membrane. This surface is a permeable support for the cultured cell layer, thus the material must be biocompatible and cell adhesive. In this work electrospun membranes were investigated: they were obtained by coupling poly(carbonate)urethane (Bionate® II 80A) conjugated with gelatin at different percentage (Bionate®:Gelatin at 50:50, 70:30, 80:20, 90:10, 100:0). The electrospinning technique was chosen to obtain porous membranes, while Bionate®'s flexibility allows mimicking the cyclic stretching of barriers in the human body (i.e. alveolar barrier during breathing).

METHODOLOGY

Selection of the membrane material

In this work a commercial poly(carbonate)urethane copolymer (Bionate® II 80A) was used to replicate the basement of the physiological barriers. Additionally, in order to increase the cell adhesion, gelatin was used in combination with Bionate® to obtain the final formulation for the membrane.

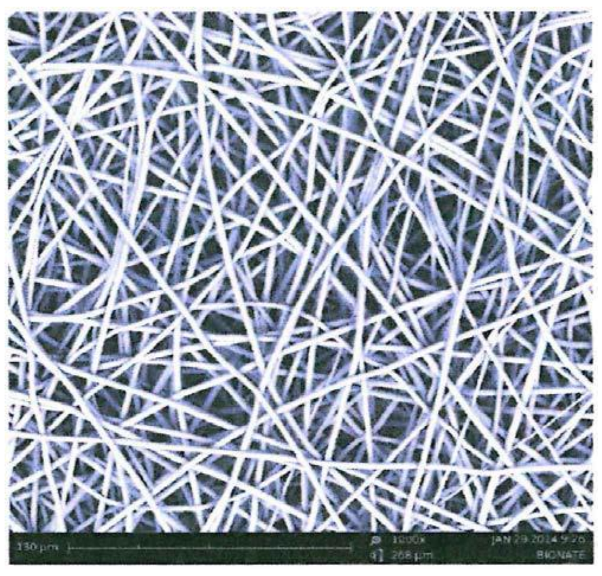

Fig. 1: SEM image of Bionate II 80A electrospun membrane. scale bar 130 µm, 1000X magnification

Membrane fabrication

The electrospinning technique was selected to obtain the porous support (Figure 1 and Figure 2). Electrospinning parameters are shown in Table I.

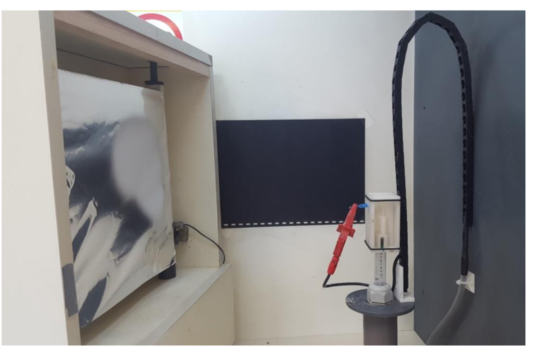

Fig. 2: Electrospinning setup

TABLE I

Voltage [KV]	Distance needle-collector [cm]	Q [mL/h]	Solvent	10%Bionate®:10%Gelatin
30	15	1	1,1,1,3,3,3 Hexafluoro-2-Propanol (HFP)	50:50 70:30 80:20 90:10 100:0

Electrospinning parameters

Mechanical properties evaluation

The Young's modulus was evaluated for the porous membranes in dry conditions (Figure 3A), in order to evaluate how gelatin can influence the stiffness. Moreover, since the membranes will be used in a rather aggressive environment and application, the mechanical characterization was repeated for long term tests, evaluating their structural properties after several days of incubation in Phosphate Buffered Saline (PBS 1X), as shown in Figure 3B.
The same experiments were performed adding a crosslinker to the gelatin before electrospinning.

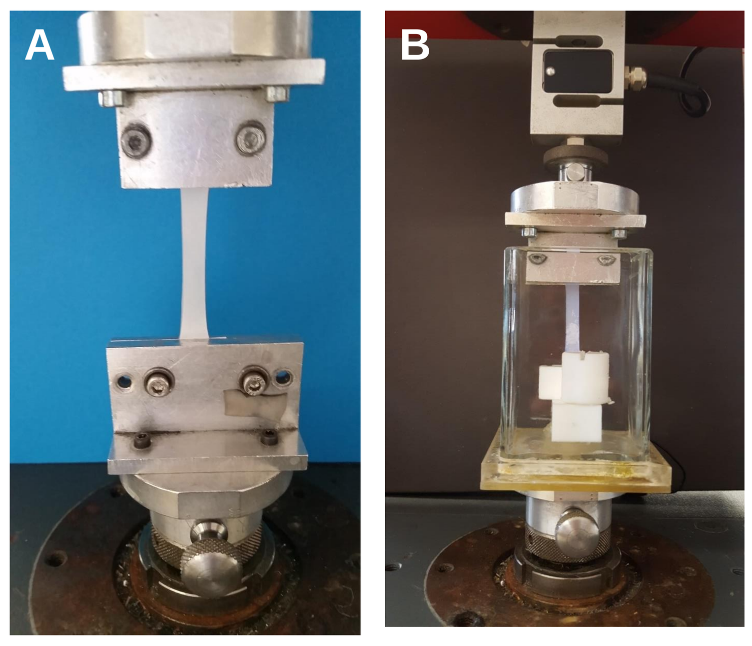

Fig. 3: Mechanical test in dry conditions (A) and wet conditions (B)

Contact angle

The contact angle was evaluated; Membranes with different percentages of gelatin were evaluated to investigate how gelatin improves wettability. Moreover, for further increasing the wettability, the membranes were dipped in 70% ethanol/distilled water (70% Eth/dH2O) and the contact angle was measured.

RESULTS

Membrane characterisation

The thickness of the electrospun membranes is 73.18±26.67 µm. Electrospun membranes were tested mechanically in dry conditions and the results are shown in Figure 4. Table II shows the Young's moduli of the membranes in dry conditions, calculated within the linear region.
Mechanical tests were repeated by immersing the samples in a PBS solution, in order to verify the possibility of hydrolytic degradation. In Figure 5 are shown the Young's Moduli of the different membranes, tested at 0, 1 and 3 days of incubation (wet conditions).
Finally, hydrophilicity was evaluated analyzing water droplet contact angle on the different membranes, evaluating how the treatment with 70% Eth/dH2O improves the wettability (Table III).

TABLE II

Bionate®:Gelatin	Young's Modulus (without crosslinker) [MPa]	Young's Modulus (with crosslinker) [MPa]
50:50	114.00±25.43 @0.4%	89.25±14.46 @1%
70:30	77.00±11.81 @0.4%	74.72±16.50 @1%
80:20	8.12±0.77 @0.6%	43.35±3.60 @1%
90:10	1.41±0.45 @5%	21.76±6.42 @3%
100:0	1.10±0.26 @5%	-

Young's Modulus within the linear region

TABLE III

Bionate®:Gelatin	Contact Angle [Degree]	Contact Angle after 70% Eth/dH2O treatment [Degree]
50:50	-	-
70:30	-	-
80:20	117.2±1.9	70.9±10.1
90:10	119.3±4.8	86.2±3.0
100:0	136.9±4.6	120.8±12.4

Contact angle of a water droplet on electrospun membrane

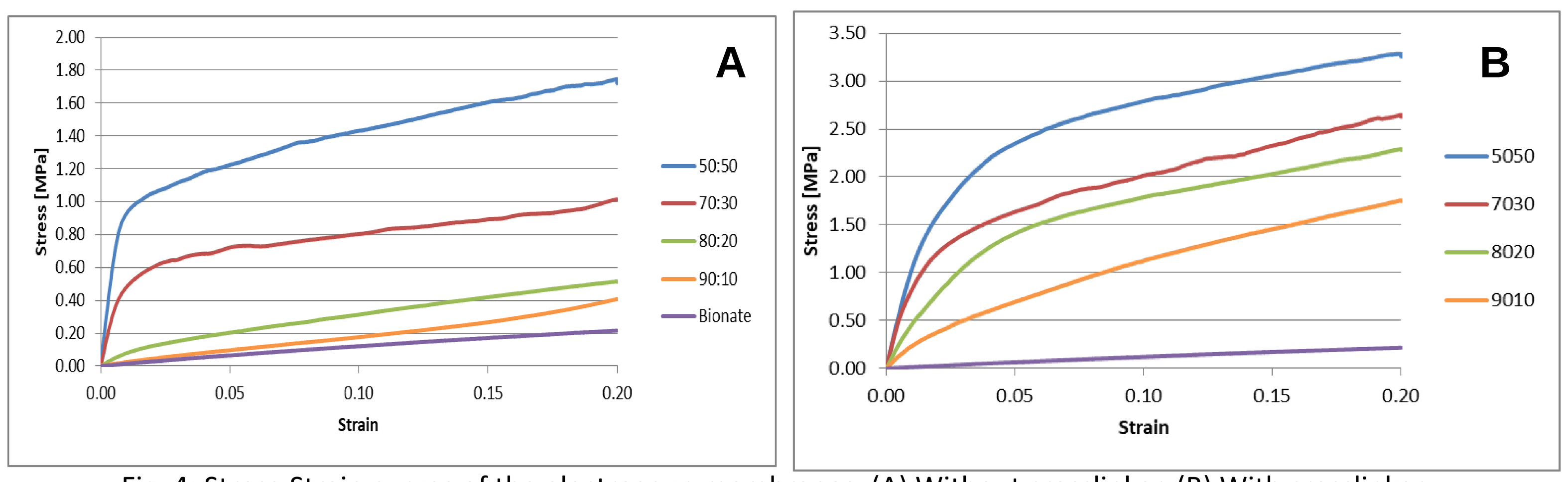

Fig. 4: Stress-Strain curves of the electrospun membranes. (A) Without crosslinker. (B) With crosslinker.

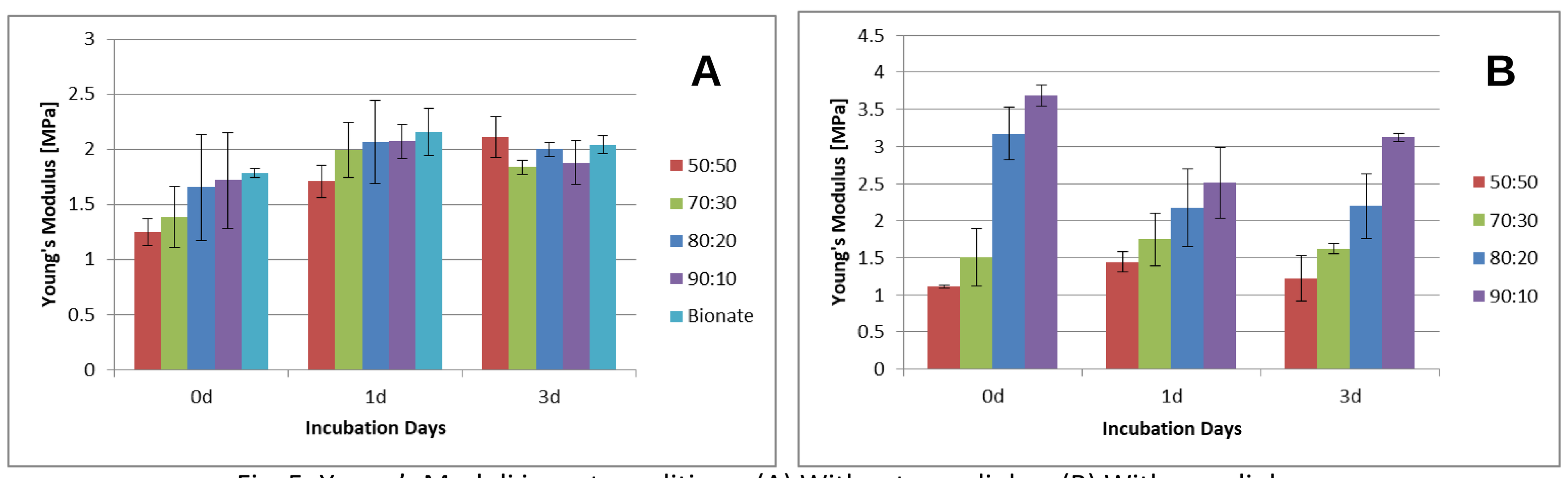

Fig. 5: Young's Moduli in wet conditions. (A) Without crosslinker. (B) With crosslinker.

CONCLUSIONS

Here we present a material that is able to replicate the basement of the physiological barriers, as it is biocompatible and it becomes hydrophilic after the treatment with 70% Eth/dH₂O.
In wet environment, all the membranes show an elastic behaviour, so they can be used in application where a flexible moving membrane is needed (i.e. alveolar barrier, intestine barrier).
In wet conditions without the addition of the crosslinker, the resulting elastic modulus of the different membranes is almost constant throughout the incubation time, suggesting that the gelatin has been removed during the incubation in PBS. However, adding the crosslinker to the gelatin, the resulting Young's moduli of the membranes increase with the decrease of the amount of gelatin for all the incubation time. This suggests that varying the amount of gelatin, it is possible to obtain membranes with different stiffness, allowing mimicking different type of physiological barriers and different pathological conditions.

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