

## **Original Research Article**

### **Polycaprolactone and polycaprolactone/ chitosan electrospun scaffolds for tissue engineering applications**

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**Abstract:** Scaffold engineering and fabrication techniques play a fundamental role in biomedical engineering applications. Tissue engineering has been considered as a very promising alternative therapeutic strategy compared to traditional approaches in order to reduce the cost of treatment for patients and cope with the need for finding suitable donors for transplantations and tissue/ organs regeneration. Several novel approaches have been employed, including the production of biological, synthetic and bio-artificial hybrid formulations that can mimic the structural, physicochemical, mechanical and biological properties of natural tissues and organs. Electrospinning, which belongs to the electro hydrodynamic techniques, has been considered as a suitable technology to fabricate non-woven fibrous formulations that could be utilized as scaffolds. In the present study, blend electrospun fibers made of polycaprolactone (PCL) and chitosan (CS), using trifluoroethanol (TFE) as an alternative solvent; have been investigated regarding their structural, physical and mechanical properties. Different scaffolds characteristics, such as average fiber diameter, porosity, density, contact angle, hydration and mechanical properties have been studied, and a comparison between PCL and PCL/ CS fibers was performed. In a nutshell, PCL/ CS fibers were thinner, more hydrophilic, exhibiting higher porosity and superior mechanical properties, compared to PCL fibers, and therefore could be considered as suitable scaffolds for tissue engineering applications.

**Keywords:** Biomedical Engineering, Blend electrospinning, Chitosan, Fibers, Polycaprolactone.

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#### **INTRODUCTION:**

Biomedical engineering involves the application of different principles of biology and engineering to develop bio-artificial substitutes for replacing damaged tissues [1-4]. Electro hydrodynamic techniques, such as electrospinning, have been used to produce scaffolds with different characteristics [5-9]. The latter mimic the porous microenvironments of the extracellular matrix (ECM) [10]. A wide variety of polymers have been used to create non-woven fibrous scaffolds with single or multi-layered structure [11-16]. In addition, the combination of different polymers can enable the production of scaffolds with enhanced structural, physicochemical and mechanical properties [17]. Polycaprolactone (PCL) and chitosan (CS) are two polymers that have been used as scaffolds materials [18, 19]. The combination of the two polymers has shown promising results in the fields of tissue engineering and drug delivery [20-23]. In this study, two different scaffolds types, made by PCL or by a blend of PCL and CS, were fabricated using the blend electrospinning method. Different characterization techniques were

used to investigate the scaffolds' morphological, structural and mechanical properties.

Polycaprolactone (PCL) (Mn 70000-90000) and chitosan (CS) (Medium MW) were purchased from Sigma-Aldrich. 2, 2, 2-trifluoroethanol (TFE) was purchased from abcr GmbH & Co.Kg. All materials were used as received.

Two different polymer solutions were prepared. For the first solution, 190mg/mL PCL was dissolved in TFE, while for the second solution 180 mg/mL PCL and 10mg/mL CS in TFE were used as a blend. 3 mL syringes were used as polymer reservoirs, equipped with blunt-tipped needles (0.6 mm, inner diameter). Electrospinning was performed for 1h, at room temperature at an accelerating voltage of 23 kV and a spinning distance of 20 cm, using a custom-made apparatus. The solution flow rate was 1 mL/h. After electrospinning, the scaffolds were removed from the collector and kept under vacuum for 12h until the remaining solvent was evaporated.

5 mm diameter disks were punched out from PCL and PCL/CS scaffolds and were coated with AU/Pd for 30s. Pictures of the samples were obtained using a scanning electron microscope (S3400N, Hitachi) under high vacuum and accelerating voltage of 15 kV, at different magnifications.

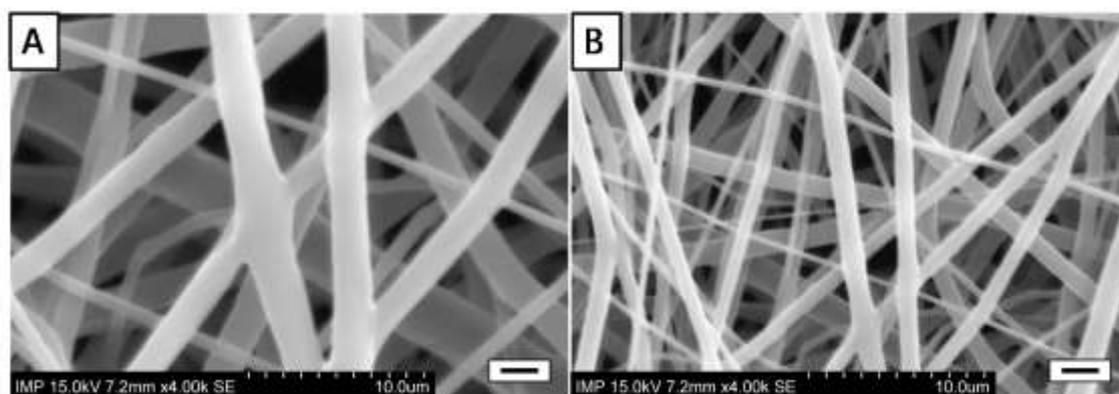
Cyclic uniaxial mechanical tests were performed using a tensile testing instrument (LM1 Test bench, BOSE), equipped with a 200 N load cell. Rectangular, 15×10 mm strips (n = 5) were punched out of the electrospun specimens and were tested at 0 - 30% strain, 1 Hz, at room temperature. The applied force and the local principal strain were monitored, and Young's modulus was calculated.

The total volume of scaffolds and the volume of the pores were calculated by the ethanol infiltration method to determine the porosity of the scaffolds [24-25]. All measurements were carried out in triplicates. Water uptake of the scaffolds was analyzed by fully hydrating the samples in PBS at 4°C, room temperature (RT) and 37°C for 1d and 7d. The rectangular specimens were weighted before ( $M_i$ ) and after ( $M_A$ ) incubation of the above mentioned periods of time and temperature. The hydrated samples weighted again and

the  $M_A$  was recorded. All measurements were carried out in triplicates. Moreover, to evaluate the surface hydrophilicity, 10 mm diameter disks were punched out from the specimens and were fixed on the holder of the contact angle instrument (FM40 Easy drop, Krüss). The static contact angle measurements were performed at room temperature using bi-distilled water. Pictures were taken 5s after 1 $\mu$ L droplet was placed at the surface of the scaffolds. Measurements were performed in quintuplicate.

#### DISCUSSION:

The SEM pictures revealed that both types of fibers had a smooth surface, cylindrical shape and no strict orientation (Figures 1A and 1B). The fibers made of PCL had an average diameter of  $1.77 \pm 0.30 \mu\text{m}$  while the PCL/CS fibers had an average diameter of  $1.09 \pm 0.43 \mu\text{m}$  and were significantly thinner ( $p < 0.001$ ). The addition of CS in the blend changed solution properties, such as electrical conductivity and viscosity [26]. As a polyelectrolyte, CS led to an increase in the electrical conductivity which has been linked with a decrease in the average diameter from previous studies [26, 27]. The polymeric jet is subjected to more intense stretching in the bending and stretching instability regions inside the electrical field during electrospinning when it is more conductive [11].



**Fig- 1 SEM pictures of electrospun fiber mats made by PCL (A) and PCL/CS (B); magnification = 4000x, scale bars = 2  $\mu\text{m}$ .**

Table 1 summarizes the average density and porosity values for both scaffold types. The average density of the PCL and PCL/CS scaffolds was  $0.18 \pm 0.05 \text{ g/cm}^3$  and  $0.12 \pm 0.05 \text{ g/cm}^3$ , respectively ( $p > 0.05$ ). In addition, the average porosity of the PCL and PCL/CS fiber mats was  $89.84 \pm 0.99 \%$  and  $92.20 \pm 0.80 \%$ , respectively ( $p < 0.05$ ). The density of the PCL/CS scaffolds was lower than the density of the PCL scaffolds while the porosity of the PCL/CS scaffolds significantly increased compared to PCL scaffolds. Fibrous scaffolds with higher porosity exhibit lower density. The density and the porosity can be modulated through optimization of the process and solution parameters [16, 24]. A change in the solution

conductivity can be accounted for the change in the aforementioned parameters [28].

The average contact angles for PCL and PCL/CS scaffolds are summarized in Table 1. PCL fibers had a contact angle of  $109.66 \pm 1.62^\circ$  while the addition of CS resulted in a significant decrease in the contact angle value, which was  $70.24 \pm 1.70^\circ$  ( $p < 0.001$ ). A more hydrophilic surface for the PCL/CS fibers is more preferable and was expected since CS is much more hydrophilic compared to PCL. The surface hydrophilicity is very important for cell adhesion and proliferation as well as for the adhesion of proteins in vivo [1, 29, 30].

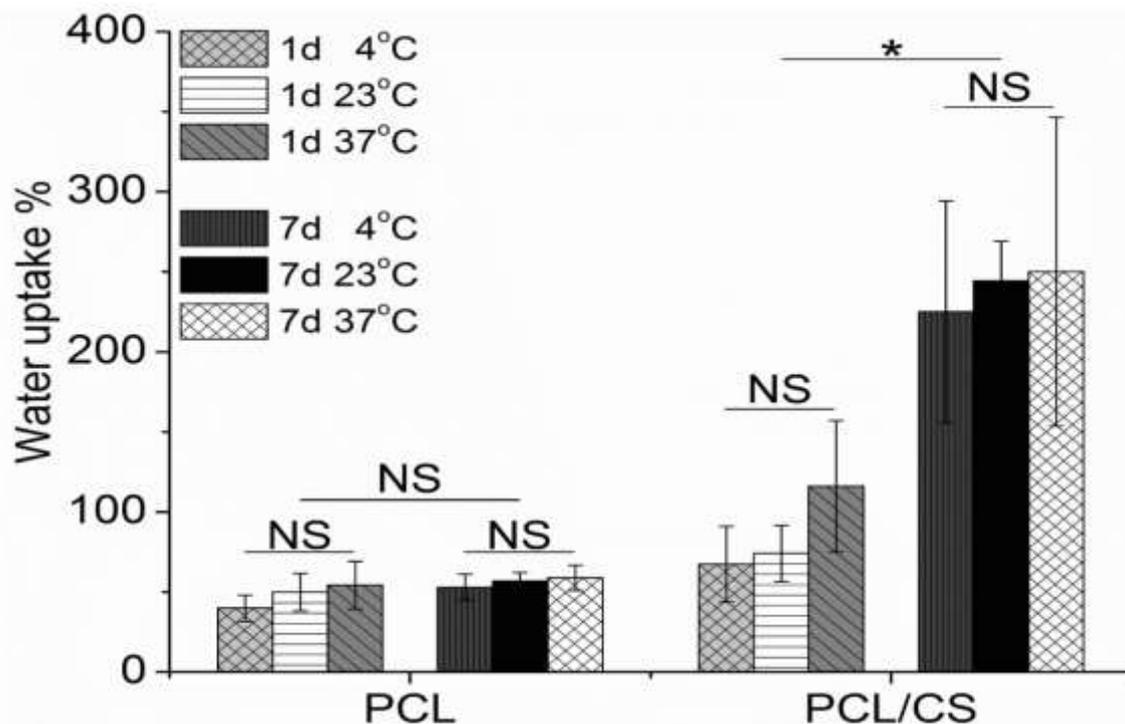
**Table 1: Summary of structural and physical properties of the electrospun PCL and PCL/CS fibers; n = 3 (density & porosity studies), n = 5 (static water contact angle measurements), mean ± SD.**

Sample	Density (g/cm <sup>3</sup> ), mean ± SD	Porosity (%), mean ± SD	Contact angle (degree), mean ± SD
PCL	0.176 ± 0.046	89.836 ± 0.985	109.66 ± 1.616
PCL/CS	0.115 ± 0.051	92.202 ± 0.796	70.24 ± 1.699

The hydration of the electrospun fibers at different temperatures and different time periods was studied (Figure 2). After one day of full immersion in PBS, the PCL scaffolds average hydration values were 39.90 ± 8.24%, 49.96 ± 11.67% and 54.34 ± 14.88%, at 4, 23 and 37 °C respectively. The average hydration values of PCL/CS scaffolds after one day were 67.36 ± 23.77%, 74.16 ± 17.53% and 116.11 ± 41.09%, at 4, 23 and 37 °C respectively. After seven days of immersion in PBS the hydration values for PCL scaffolds were 52.85 ± 8.17%, 56.85 ± 5.27% and 58.83 ± 8.01%, at 4, 23 and 37 °C respectively, while the values for PCL/CS scaffolds were 225.12 ± 69.27%, 244.4 ± 24.73% and 250.17 ± 96.54%, at 4, 23 and 37 °C respectively. The temperature in which the scaffolds were maintained had no significant effect on the average hydration level for neither types of scaffolds (p>0.05). However, a trend of increasing values of hydration due to an increase in temperature was observed in all cases (Figure 2). Faster penetration of water in the scaffolds is achieved through higher encroachment of the hot water molecules [24]. The addition of CS resulted in an increase of the

average hydration of scaffolds PCL/CS compared to PCL scaffolds, regardless of the temperature or the immersion period. That was expected because of the increased hydrophilicity and porosity of the PCL/CS scaffolds that was previously described. Finally, the time period of the immersion of the scaffolds in PBS differed significantly only for PCL/CS fibers (p<0.05), in contrast to PCL fibers where it had a minor effect (p>0.05). The latter can be explained by the hydrophobic nature of PCL and its slow biodegradation rate [2]. Nevertheless, in both scaffold types longer immersion period led to higher hydration values.

Young’s modulus of the electrospun fibers was investigated performing tensile mechanical tests. The average Young’s modulus values for PCL and PCL/CS scaffolds were 43.27 ± 6.51 MPa and 49.36 ± 3.68 MPa, respectively. From the obtained data, it can be concluded that the addition of CS in the polymeric blend led to a significant increase of Young’s modulus (p<0.05). The latter can be correlated with altered microstructure that the addition of CS induced [31-33].



**Fig-2: Water uptake of PCL and PCL/CS scaffolds at three different temperatures (4, 23, 37 °C) and after two different time periods (1d and 7d); n = 3, mean ± SD, (\* = p<0.05, NS = p>0.05).**

## CONCLUSION:

Taken together, these preliminary studies indicate that PCL/ CS fibers could be considered suitable scaffold candidates. Future research will focus on cell seeded electrospun scaffolds with different types of cells that could be applied for tissue engineering applications.

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