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Surface modification of poly(ε-caprolactone) electrospun fibers with bone powder by DBD

Marcos Rodrigues Oliveira[1], Lauriene Gonçalves da Luz Silva[2]*, Brenda Jakellinny de Sousa Nolêto[2], Gabriely Gonçalves Lima[2], Renan Matos Monção[2], Marcos Cristino de Sousa Brito[2], Lucas Pereira da Silva[2], Ediones Maciel de Sousa[3], Thercio Henrique de Carvalho Costa[4], Fernanda Roberta Marciano[2] and Rômulo Ribeiro Magalhães de Sousa[1,2]

[1]Departamento de Engenharia Mecânica, Universidade Federal do Piauí, Teresina, PI, Brasil
[2]Programa de Pós-graduação em Engenharia e Ciência dos Materiais, Universidade Federal do Piauí, Teresina, PI, Brasil
[3]Programa de Pós-graduação em Física, Universidade Federal do Piauí, Teresina, PI, Brasil
[4]Departamento de Engenharia Mecânica, Universidade Federal do Rio Grande do Norte, Natal, RN, Brasil
**lauriene@ufpi.edu.br*

Abstract

Poly(ε-caprolactone) (PCL) is a biodegradable polyester with promising properties in tissue engineering, particularly in the creation of living structures for regenerative medicine. This study focused on the surface properties of electrospun PCL membranes combined with bone powder, treated using Dielectric Barrier Discharge (DBD) plasma in argon and atmospheric air environments. The electrospinning technique was employed for its ability to produce fibrous scaffolds that mimic the extracellular matrix, enhancing cell adhesion and proliferation. Scanning Electron Microscopy (SEM) results indicated that the morphology of the electrospun samples remained unchanged, exhibiting random fiber orientation and the presence of hydroxyapatite, although it was not fully incorporated. Infrared spectroscopy confirmed the characteristic polymer groups, and contact angle measurements demonstrated the hydrophobic nature of the films. However, increasing the plasma exposure did not entirely convert the surface to a hydrophilic state.

Keywords: *biological, plasma, poly(ε-caprolactone), surface modification.*

Data Availability: *Research data is available upon request from the corresponding author.*

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1. Introduction

Tissue engineering is emerging as a promising field of research, paving the way for the creation of living structures capable of transforming regenerative medicine. Its central aim is to build functional substitutes for damaged tissues or organs, restoring health and well-being to patients[1-3].

The biodegradable aliphatic polyester, poly(ε-caprolactone) (PCL), has promising potential in tissue engineering and drug delivery applications, thanks to its remarkable mechanical and structural properties. The surface property of the material is a crucial factor in cell adhesion, influencing the behavior of cells during seeding and growth. Understanding the different properties and their effects is fundamental to the development of biocompatible and effective materials for various applications in tissue engineering and regenerative medicine[4].

However, their low wettability and surface energy characteristics have an adverse impact on cell attachment and proliferation[5,6]. It is therefore imperative to modify the surface properties of PCL by introducing additional functional groups onto its surface. Surface modification by atmospheric pressure plasma has emerged as an innovative technique in recent years. Its ability to create and/or improve topographical and functional characteristics of surfaces makes it ideal for improving the biocompatibility of biomaterials[7,8].

The treatment with DBD plasma is widely used to modify the surface properties of scaffolds, providing specific functionalities such as increased hydrophilicity and enhanced adhesion of bioactive molecules to the surface. PCL is recognized for its biocompatibility with various types of drugs, enabling uniform drug distribution, while its long-term degradation allows controlled drug release over several months, especially in applications focused on bone tissue engineering (BTE)[9,10].

Therefore, to produce scaffolds that mimic the extracellular matrix, the electrospinning technique was employed. Additionally, it is important to note that the

objective of the work is to use PCL as a support for the release of bovine bone powder, which simulates synthetic hydroxyapatite and exhibits a biological composition similar to that of natural bone.

The electrospinning technique also known as electrostatic spinning, is widely used in the production of scaffolds that form mats and membranes with large surface area and high porosity. These are often employed as structural supports for tissue fixation and regeneration, owing 3D dimensional structure that mimics the extracellular matrix, facilitating cell adhesion, migration, and proliferation[10,11]

In this work, the electrospinning technique was used due to its simplicity and effectiveness in manufacturing nanofibers. The aim of this study was to produce fibrous membranes using the electrospinning technique, with PCL and demineralized bone powder, and to treat them by plasma at medium pressure, using a dielectric barrier discharge (DBD) with varying treatment times and discharge gases (argon and atmospheric air). Changes in surface properties were studied using scanning electron microscopy, energy dispersive spectroscopy, Fourier transform infrared spectroscopy and water contact angle analysis.

2. Materials and Methods

2.1 Materials

Polycaprolactone (PCL, Mn 80,000) from SIGMA-Aldrich, Dichloromethane (DCM) Dimethylformamide (DMF), Acetone, Absolute alcohol (NEON Inc. São Paulo). Bovine bone pieces obtained commercially.

2.2 Synthesis of bovine bone matrix

Pieces of bovine bone were purchased commercially and taken to the laboratory for a cleaning procedure using a scalpel to remove meat and fat from the bone. The bone was then boiled for 1 hour in water and salt (NaCl), and this process was repeated until no fat or impurities remained in the water. The bone was dried in an oven at 100 °C. With the aid of a toothed saw, the material was sanded to obtain the bone in powder form and then separated into particle sizes smaller than 400 mesh using an analytical sieve.

To avoid contamination, the bone powder obtained was added to absolute alcohol under heating in a chapel to speed up the evaporation process of the solution (absolute alcohol). The material was subjected to a solution based on acetone and distilled water (1:1 solution ratio v:v), stirred vigorously for 2 hours and filtered to remove the organic part of the bovine matrix. The powder was placed in a Petri dish and dried in an oven at 100 °C or on a hot plate at 80 °C. Finally, it was sieved through a 200 mesh sieve[9].

2.3 Manufacture of PCL fibers/bone powder

The polymer solution of poly (ε-caprolactone) 15 wt% and bone powder was prepared using the solvents dichloromethane and N, N-Dimethylformamide (DMF), in proportions of 5:6 and 1:6 (w:v), respectively. The PCL was dissolved at room temperature and under controlled magnetic stirring in dichloromethane for 2 hours until the polymer was completely dissolved. Then 3% bone powder and the DMF solvent were added to the polymer solution, followed by magnetic stirring for 2 hours, for incorporation into the polymer. For the electrospinning process, the distance from the tip of the nozzle to the collector static was 100 mm and the variation in electrical potential between the nozzle and the collector was 12.5-13 kV. Finally, the flow rate of the solution was controlled by an infusion pump (kdScientific, Model KDS-100) at a flow rate of 1 mL h^{-1}. The PCL fibers/bone powder were collected in a grounded collector at 22 ± 1 °C, 48-50% relative humidity.

2.4 Dielectric barrier discharge configuration

It consists of a quartz tube (110 mm long, with an internal diameter of 75 mm and a wall thickness of 2.5 mm) sealed by two PTFE (polytetrafluoroethylene) flanges in which two electrodes are housed. The upper electrode (40 mm in diameter) is anodically polarized and the lower electrode (30 mm in diameter) is cathodically polarized. The cathode is immediately above an alumina 56 mm in diameter and 2 mm thick, which acts as the dielectric in which the samples are placed. The tube is inserted 5 mm deep into the PTFE pieces. The anode is moved by a 0.1 mm precision ratchet that varies the distance between the electrodes by up to 100 mm. In addition to the reactor, a power supply was used, allowing the applied voltage to be varied from 0 to 20 kV and frequency from 200 Hz to 1.0 kHz, and an oscilloscope to monitor the treatments. The treatment conditions and sample nomenclature are shown in Table 1.

2.5 Characterizations

The surface topography of the PCL electrospun materials was analyzed before and after exposure to plasma using a scanning electron microscope (FEI COMPANY, model QUANTA FEG 250) with an acceleration voltage of 1 to 30 kV and corresponding elemental analysis by energy dispersive spectroscopy (Bruker, model Quantax EDS).

The samples were characterized in order to identify the characteristic bands of each group using Fourier Transform Infrared Spectroscopy by ATR (VERTEX 70 BRUKER). The FTIR spectrometer, equipped with a germanium prism, performs 128 scans with a resolution of 4 cm^{-1}. The spectra were obtained in the 600-3250 cm^{-1} range.

The contact angle of the samples was analyzed at room temperature using a drop of 2 μL of distilled water placed on the reference and treated substrates. The image of the drop was stored using a camera. Surftens software was used to measure the contact angles of each slide. Three measurements were taken to obtain the mean and standard deviation.

Table 1. Treatment conditions.

Samples	Treatment atmosphere	Time (min)
Base	-	-
Atm15	Atmospheric air	15
Atm30	Atmospheric air	30
Atm45	Atmospheric air	45
Ar15	Argon	15
Ar30	Argon	30
Ar45	Argon	45

3. Results and Discussions

The effect of DBD plasma treatment of atmospheric air and argon on surface morphology is examined and analyzed using scanning electron micrographs (Figure 1).

The morphology of the samples indicates interconnected and randomly oriented fibers. The resulting fibrous structures consist of distinct cylindrical fibers that are entangled with each other, the electrospinning of PCL resulted in continuous and smooth fibers, with the presence of some granules. The SEM images showed no noticeable differences. These images clearly reveal that the surface topographies are maintained on the plasma-treated samples, as there is no obvious surface damage visible.

EDS measurements were carried out on untreated and plasma-treated samples to determine the effect that plasma treatment had on the chemical composition of the surface of the PCL/bone powder samples. The results are summarized in Table 2.

The bone powder has the elemental formula $Ca_{10}(PO_4)_6(OH)_2$, and it is possible to observe the presence of the elements calcium and phosphorus, which indicates that the points with the highest light intensity in the backscattered electron micrographs are hydroxyapatite crystals that have not been fully incorporated into the material.

From Table 1, it can be inferred that after plasma treatment in both atmospheres, there is an increase in the oxygen content of the samples, due to the incorporation of oxygen, the relative carbon content also decreases with plasma treatment[10].

Table 2. Atomic compositions (%) of the untreated and plasma-treated samples.

Samples	C	O	Ca	P
Base	74.82±0.00	22.85±0.00	1.54±0.00	0.79±0.00
Atm15	79.00±8.38	20.84±3.53	0.12±0.04	0.04±0.03
Atm30	77.13±8.19	22.74±3.80	0.08±0.04	0.05±0.03
Atm45	75.88±8.03	23.75±3.94	0.34±0.07	0.02±0.00
Ar15	76.32±7.97	23.63±3.79	0.03±0.03	0.03±0.03
Ar30	76.64±8.09	23.22±3.83	0.10±0.04	0.05±0.03
Ar45	77.10±8.12	22.82±3.75	0.06±0.04	0.03±0.03

The FTIR spectra obtained made it possible to determine the molecular composition of the PCL fibers with bone powder and of the samples after dielectric barrier discharge. The results are shown in Figure 2.

The presence of carbonyl groups (C=O) is an indicator of the presence of PCL in the sample. The C=O stretching peak is a strong, well-defined peak that occurs at 1726 and 2359 cm^{-1}. The presence of methyl groups (CH_3) at 2947 cm^{-1} and methylene (CH_2) at 1049, 2873 cm^{-1} indicates the presence of ethylene glycol side chains in the PCL molecule. The COC crystalline elongation peaks occur at 1365 and 1469 cm^{-1}, the intensity of these peaks is a measure of the crystallinity of poly (ε-caprolactone) and vibration bands at 1296, 1244, 1176, 1106, 959, 731, 658 referring to the asymmetric elongation of the COC group[11-13].

Plasma treatment is a versatile technique that can significantly modify the surface properties of materials. One of the effects observed is an increase in the intensity of the oxygen-related peaks (O-H and C-O)[14], another factor is that treatment in inert gases, such as argon, introduces oxygen to the polymer surface due to the post-plasma exposure of the samples to atmospheric oxygen[15].

The contact angle values of the samples are shown in Figure 3. The results show the hydrophobicity of the surface of the samples. The small difference in values between the samples can be attributed to variations in the morphology and diameter of the electrospun fibers, factors that have a significant influence on surface roughness[16].

Depending on the intended purpose, plasma treatments can impart hydrophobicity to the surface, evidenced by contact angles equal to or higher than 90 °, as appropriate for the application[17].

With increased exposure to plasma, the contact angle results increased significantly compared to the control. Since the treatment was not as effective, it showed low capacity to increase wettability even at high treatment durations, a

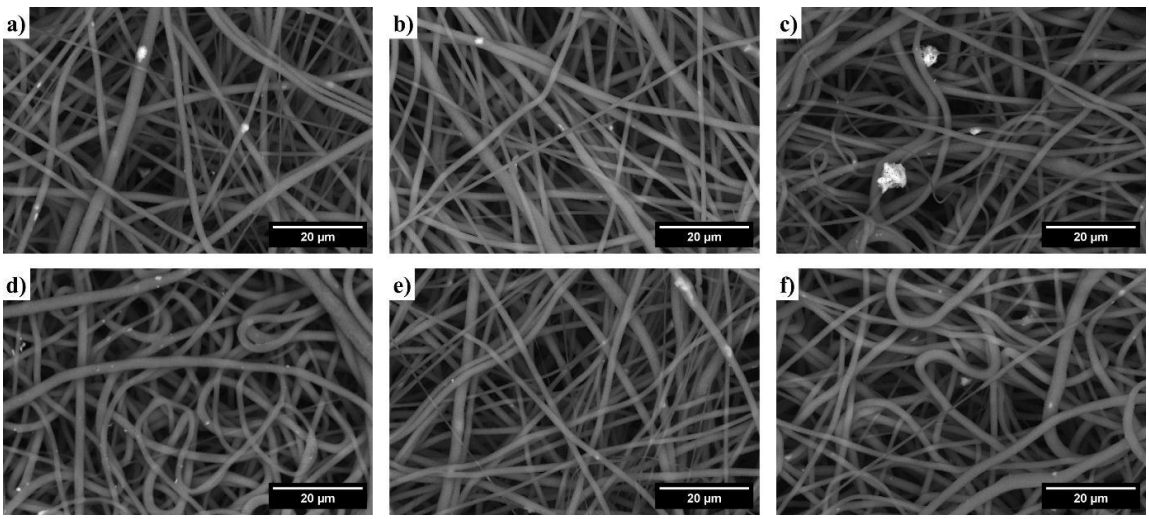

Figure 1. Micrographs of the samples after plasma treatments (5000x): (a) Atm15, (b), Atm30, (c) Atm45, (d) Ar15, (e) Ar30 and (f) Ar45.

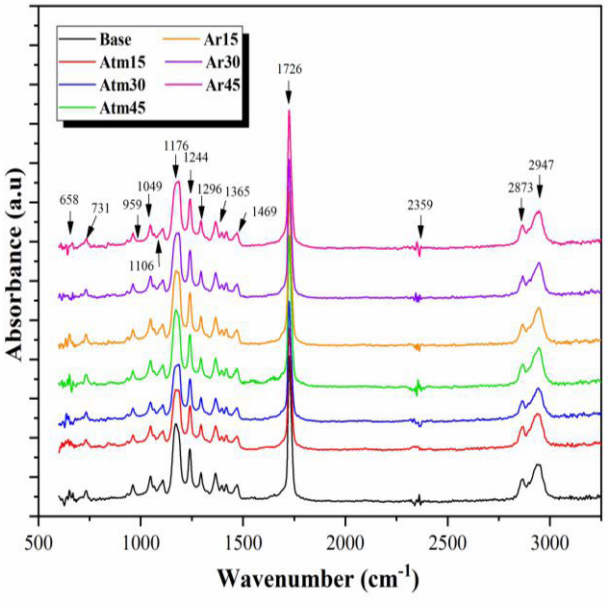

Figure 2. FTIR spectra of the base material and after treatment.

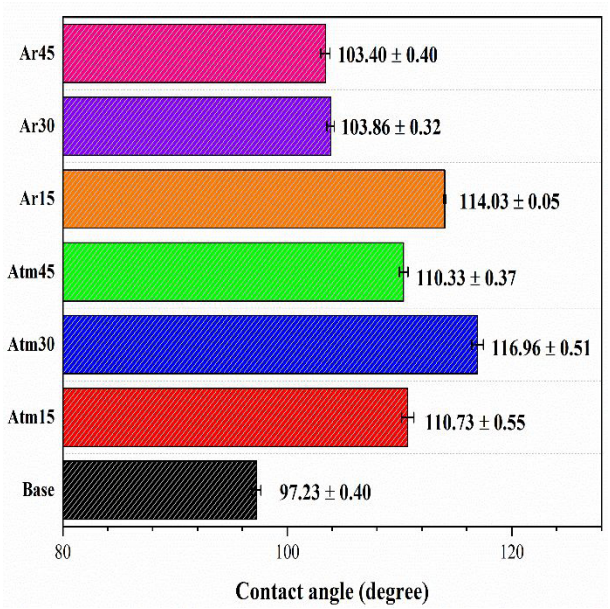

Figure 3. Contact angle of the base and treated samples.

result also found by Ozkan and Turkoglu Sasmazel[8] . This means that, under the conditions studied, plasma treatment did not completely alter the hydrophilic nature of the films.

4. Conclusions

This study explored the application of the electrospinning technique in the production of fibrous membranes using polycaprolactone (PCL) and demineralized bone powder, followed by medium-pressure plasma treatment using a dielectric barrier discharge (DBD).

The results of the morphology of the samples showed the formation of interconnected, randomly oriented fibers, with the electrospinning of the PCL resulting in continuous, smooth fibers. Plasma treatment caused no noticeable damage to the surface topography, indicating the robustness of this approach. The presence of not fully incorporated hydroxyapatite, as evidenced by elemental analysis, highlights the need to optimize the integration of demineralized bone powder into the membranes.

Analysis of the chemical composition revealed an increase in the oxygen content of the samples after plasma treatment,

indicating the incorporation of oxygenated groups. Infrared spectra confirmed the presence of groups characteristic of PCL, with plasma treatment increasing the intensity of the oxygen-related peaks.

The results of the contact angles indicated the hydrophobicity of the surface of the samples, with a significant increase after exposure to plasma. However, even at high treatment durations, the hydrophilic character was not completely altered, suggesting the need to optimize the treatment conditions.

5. Author's Contribution

- **Conceptualization –** Rômulo Ribeiro Magalhães de Sousa.
- **Data curation –** NA.
- **Formal analysis –** NA.
- **Funding acquisition –** NA.
- **Investigation –** Renan Matos Monção; Ediones Maciel de Sousa; Brenda Jakellinny de Sousa Nolêto.
- **Methodology –** Marcos Rodrigues Oliveira; Brenda Jakellinny de Sousa Nolêto; Gabriely Gonçalves Lima; Lucas Pereira da Silva.
- **Project administration –** NA.
- **Resources –** NA.
- **Software –** NA.
- **Supervision –** Rômulo Ribeiro Magalhães de Sousa; Fernanda Roberta Marciano; Thercio Henrique de Carvalho Costa.
- **Validation –** NA.
- **Visualization –** NA.
- **Writing – original draft –** Lauriene Gonçalves da Luz Silva.
- **Writing – review & editing –** Marcos Cristino de Sousa Brito.

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