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PCL scaffolds with collagen bioactivator for applications in Tissue Engineering

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Abstract

The need for human tissues is fundamental in certain genetic diseases or accidents of everyday life, they are a primary source for public health. In recent years, to alleviate these effects there was an improvement in the development of tissue engineering, scaffolds or matrices, to support an adequate mechanical and biological environment necessary for the regeneration of damaged tissues. These scaffolds are structures allowing adhesion, differentiation, cell proliferation and the supply of nutrients for growing cells.

This paper aims to obtain PCL scaffolds bioactivators, to use as support matrices for the development of human tissues, as PCL is a biomaterial widely used in the biomedical field, due to its biocompatibility, mechanical strength and biodegradability. However, this biomaterial is hydrophobic, which means that there are difficulties in cell adhesion. To solve this problem, a coating of PCL scaffolds with collagen was used, since collagen is a hydrophilic protein existing in the formation of various tissues, so improves cell adhesion.

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Keywords: Biocompatibility; Collagen; Poly(ϵ -caprolactone); Scaffolds; Tissue Engineering

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1. Introduction

Tissue Engineering is an interdisciplinary field that necessitates the combined effort of cell biologists, engineers, material scientist, mathematicians, geneticists, and clinicians toward the development of biological substitutes to restore, maintain, or improve tissue function (Atala, 2007).

Tissue engineering has emerged as a result of the shortage of organs for transplantation. Malfunction or loss of total or partial functions of an organ or tissue resulting from disease or injury is currently one of the most important and disturbing public health problem, affecting a significant number of people around the world (Bártolo *et al.*).

Scaffolds are support structures used in tissue engineering to provide the three-dimensional growth of cells in an organized way (Hoque *et al.*, 2008) (Domingues *et al.*, 2009), they made of biodegradable materials, the most common biopolymers. The development of biodegradable polymers has been considered of great interest for biomedical applications (Cheng *et al.*, 2004) (Wu *et al.*, 2006).

The biocompatibility and good polymer-cell interaction depend of primarily cell adhesion to the substrate. Only after adherent cells begin its process of proliferation, it starts division and production of a new extracellular matrix. Additionally, the porosity and surface roughness benefit from the adsorption of proteins present in the culture medium, in order to measure the cell adhesion (Barbanti *et al.*, 2004).

Several works show that there is a close relationship between hidrophilicity and cell adhesion. The hydrophilic substrates tend to allow better interaction with cells, within certain parameters. However, synthetic polymers often require a selective modification of the surface, for hydrophilicity, biocompatibility and cell adhesion (Zhang *et al.*, 2001).

This paper aims to obtain PCL scaffold bioactivators, to use as support matrices for the development of human tissues, as PCL is a biomaterial widely used in the biomedical field, due to its biocompatibility, mechanical strength and biodegradability. The PCL used in the tests was Poly (ϵ -caprolactona) (PCL) with Mw 50.000 (CAPA 6500), obtained from Perstorp Caprolactones Cheshire, United Kingdom). The collagen “Calf Skin” used for the bioactive of scaffolds was purchased from Calbiochem.

2. Methods

2.1. Scaffold Design and Fabrication

The BioExtruder device, developed by the Centre for Rapid and Sustainable Product Development of the Polytechnic Institute of Leiria, was used to fabricate the 3D porous scaffolds.

The deposition strategy (raster deposition strategy), scanning velocity, and filament distance for each layer were directly programmed through the BioExtruder scanning deposition generator routine, which was developed based on the ISO programming language (Table 1). PCL scaffolds have a geometry of 0/90°.

Table 1. Processing Conditions of scaffolds.

Process	Value
Reservoir temperature	80°C
Reservoir pressure	4 bar
Screw velocity	15 rpm
Extrusion chamber temperature	70°C
Extrusion nozzle diameter	0.30 m

2.2. Bioactivation of PCL scaffolds with collagen

The surfaces of the PCL scaffolds were initially modified by the plasma processing equipment for plasma model called Femto Diener Electronic. In Table 2, it can be observed the parameters used for plasma treatment device.

Table 2. Processing Conditions of plasma AR.

Conditions	Value
Power	100 W
Time	30s
Frequency	0,6Hz

To the polymerization of acrylic acid into the surface of PCL scaffolds, a solution of acrylic acid was used to which it was added 0.0150 mol/l of vitamin B2 in a ratio of 1:20, to reduce the dissolved oxygen. The scaffolds were exposed to UV radiation for 30 minutes, and afterwards accommodated in a Petri dish. After exposure to UV radiation, the scaffolds were washed with distilled water to remove the excess homopolymer and placed to dry in a vacuum desiccator (Cheng, et al., 2004).

After modifying the surface of scaffolds with AAc, an immobilization of collagen was carried out. This process consists of the immersion of the scaffolds (PCL-AAc) into PBS (phosphate buffered saline), containing 5 mg/ml carbodiimide water-soluble (1-ethyl-3(3-dimethylaminopropyl), during 1 hour, in a temperature of 4.° C to activate the carboxyl groups on the surface of PCL scaffolds. After this process, the incorporation of collagen scaffolds in concentration 0.5 mg/ml for 5 hours was carried out. The scaffolds were then washed in PBS for 1 hour at room temperature, to remove the collagen that is physically adsorbed on the surface of the scaffold. The PCL collagen immobilized (PCL-Col) scaffolds were then dried under reduced pressure, and stored in a refrigerator (Cheng, et al., 2004).

2.3. Morphology Analysis – SEM

The Scanning electron microscope (SEM) was used to investigate the surface morphology of the PCL scaffolds, modified by the acrylic acid through polymerization and immobilization of collagen. In this tests, the samples were fixed in a metal and coated with a thin golden layer, using the equipment Edwards EXC 120. The samples were observed using the equipment mark Jeol model JSM-5310.

2.4. Chemical Analysis – FTIR

The Fourier Transform Infrared Spectroscopy (FTIR) is a technique to obtain spectra of the absorption emission. Using this technique, we can observe the constitution of chemical scaffolds. Infrared absorption spectra of the PCL and PCL-COL scaffolds were obtained from a spectrophotometry model Alpha-p of Brucker. For each spectrum were performed a total of 3 scans.

2.5. Water Contact Angle

This analysis shows the change in hydrophilicity / hydrophobicity or wettability of the sample, before and after the process of grafting of carboxyl functional groups and immobilization of collagen. The measures were performed by Contact Angle System (OCA) dataphysics. To carry out these measures, it was used distilled water. For this work, PCL films were made. The PCL was dissolved in chloroform (6% w/w) and placed in petri dishes. The films were dried by the method of solvent evaporation at room temperature.

2.6. Biological Behavior (MTT)

In this work, normal human fibroblasts were used, provided by the Faculty of Medicine, University of Coimbra, Portugal. The culture medium was AMH - F10 (Gibco) with fetal calf serum (FSC) to 15% (Fetal Bovine Serum / Heat inactivated, Gibco). Flasks containing 500 ml of this medium were added to the following antibiotics: 5 ml of penicillin and streptomycin 10mg/ml (Sigma), 5 ml of L-glutamine (Sigma) and 1 ml of kanamycin (Sigma). All reagents were sterile. Adhesion tests followed ISO 10993. During this work, dishes of 24 wells were used, which cut up the scaffolds with a diameter of 1 cm.

The cell culture used was 7×10^4 cells/ml. The dishes were isolated from the culture incubator at 37 °C and 5% CO₂, after sprayed with alcohol at 75°.

The MTT colorimetric assay is a more quantitative one used in live mammalian cells and its proliferation. To this test, it was added 360 µl of new medium and 40 µl of methyl 3 - (4,5-DimethylTiazol-2-yl) -2,5-diphenyl tetrazolium (MTT) (Sigma-Aldrich) to each sample, in a petri dish to incubate during 3 to 4 hours at a temperature of 37°C, in 5% CO₂ atmosphere.

After incubation, the previous solution was withdrawn from the petri dish, and added 400 µl of acid isopropanol cooled to 4°C for each sample, leaving on for 15 minutes at room temperature.

3. Results and Discussion

3.1. Morphology Analysis

The morphological evaluation of the structures was carried out using the Scanning Electron Microscopy (SEM). The scaffolds produced with the Bioextruder present a well-defined internal geometry with square interconnected pores of regular dimensions (600 x 600 µm) and uniform distribution. The extruded filaments show a regular circular geometry with 350 µm diameter.

Figure 1 shows the images of the surface PCL scaffolds and the PCL scaffolds with immobilization of collagen. The comparison of the SEM of the PCL scaffolds and the PCL-Col ones show the formation of a coating on the scaffold filaments when collagen is added.

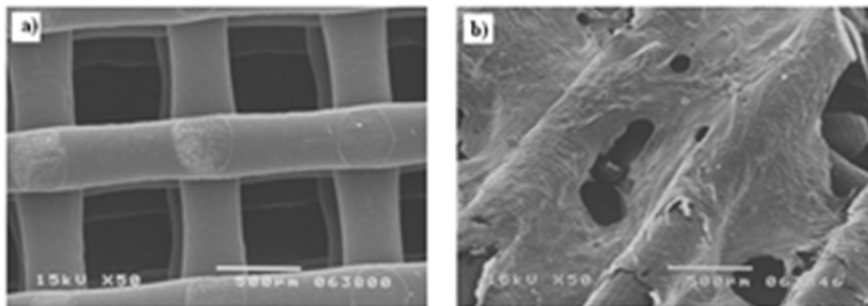


Fig. 1. SEM images, a) PCL scaffold, b) PCL-Col scaffold.

3.2. Chemical Analysis

The FTIR Analysis to a PCL 0/90° scaffold shows that the collagen immobilization was adsorbed by the filament scaffold, since the spectrum of the bands are similar between the PCL and collagen (Fig. 2).

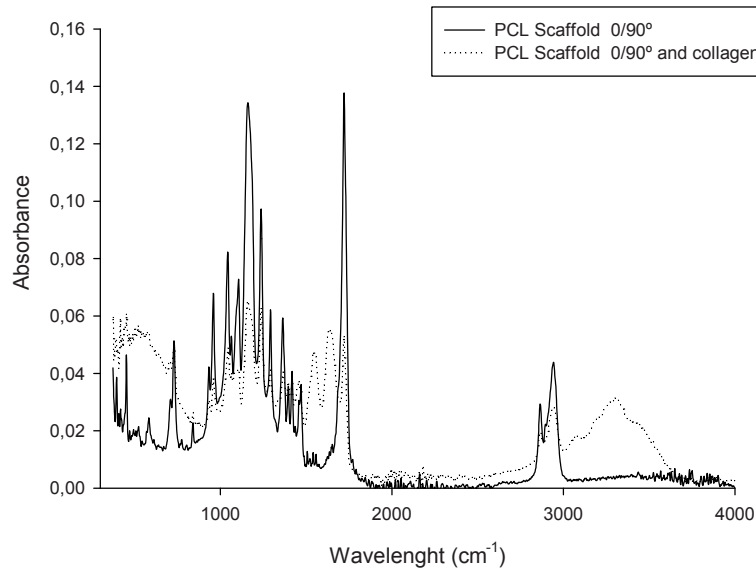


Fig. 2. FTIR spectrum of PCL scaffold 0/90°

The PCL is an aliphatic polyester. The main links present in the polyester are C = O, C – O – O and C – C. In the spectrum of the PCL scaffold, the absorption peak 1750 cm^{-1} corresponds to C = O bonds C – O – C bands, which are represented by $\sim 1200 \text{ cm}^{-1}$ band at $\sim 2995 \text{ cm}^{-1}$ corresponding to the bond C – H.

The presence of the bands 1628,73 cm^{-1} (amine I), 1544,4 cm^{-1} (amine II) and 1406,22 cm^{-1} (amine III) correspond to the amines composing the collagen molecules, which seems to corroborate that the PCL scaffolds with collagen are bioactivators.

3.3. Water Contact Angle

Given the values of the contact angle obtained during the tests, it can be concluded that the incorporation of collagen into the PCL films made them more hydrophilic, as there is a decrease in the contact angle.

The water contact angles were measured to compare the hydrophilicity of modified and unmodified film surfaces.. The water contact angle of the pristine PCL film was 59,15°. After collagen immobilization, the water contact angle of the film initially dropped to 46,15° (Table 3).

Table 3. Water contact angle

Material	Value
PCL film	59,15 ± 0,26
PCL/Col film	46,29 ± 0,20

3.4. Biological Behavior

The results obtained after a 7 day cell culture show that the scaffolds coated with collagen has a better cell adherence to PCL scaffolds, due to the hydrophilicity of coated scaffolds as observed in the contact angle tests. In 14 days of culture, the scaffolds coated with collagen present a decrease in cell adhesion, due to cell death as cells come into confluence (Fig.3).

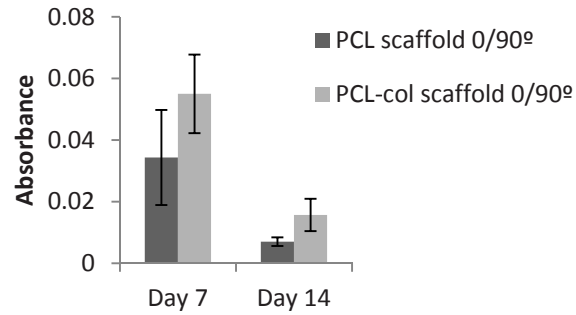


Fig. 3. Viable fibroblasts cells on PCL scaffolds and PCL-Col scaffolds

4. Conclusions

The Bioextruder Systems enables to obtain three-dimensional arrays, where the pore size diameters of the filaments can be controlled, ensuring a good cell adhesion.

The hydrophobic character of PCL, which disrupt cell adhesion, does not prevent its use in biomedical applications, as the PCL modified with collagen allows for increased cell adherence. Apart from this, collagen is a component of several human tissues well accepted by the body.

Results show that the method of surface modification used in this work allows the collagen immobilization on the surface of PCL scaffolds, which suggest that PCL-Col scaffolds can be applied in tissue engineering and regenerative medicine.

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