TISSULAR SUBSTITUTES COMPOSED BY FIBER PCL

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Abstract*. Burns present high clinical complexity after the loss of body defense and activation of inflammatory cascade. Complications such as Multiple Organ Dysfunction Syndromes (MODS) reflect the need to improve techniques used in its therapy. In addition to preventive actions, tissue engineering is a viable alternative for mimicking the extracellular matrix through biocompatible materials.* ***Objective:*** *To develop a synthetic tissue using a polymeric matrix.* ***Materials and Methods:*** *For the manufacture of scaffolds, poly (ε-Caprolactone) [PCL] and reagents were used to form the solution which, after rotary jet spinning, was crosslinked. Following technical standards, biological and material characterization was performed.* ***Results:*** *The solutions showed homogenization and the FTIR confirmed the material employed.* ***Conclusion:*** *Effective jet spinning of non-toxic scaffolds were obtained.*

Keywords*: Burns, Biocompatible Materials, Rotary jet spinning.*

1. INTRODUCTION

Burns are a public health problem. Considering its high clinical complexity, it is characterized by prolonged hospitalizations; susceptibility in the immune system; high morbidity and mortality [ABTO, 2018; WHO, 2017].

For survival, the gold standard in treatment is skin transplantation, because the human organism has limitations in the process of tissue repair, depending on the magnitude of the damage [BRITO, 2016; INTO, 2017].

Nevertheless, the disparity between effective organ donors and recipients mobilized the entire complex National Transplant System (TNS). Therapeutic alternatives for changes in this scenario are being created and/or improved [BRITO, 2016; INTO, 2017; PAGGIARO et al., 2017].

Among emerging technologies, biomaterials are tissue engineering examples that aim to repair or replace tissue through the structure-function relationship in normal and diseased tissues [PARK, 1984; BRAZIL, 2011; TENORIUM, MELLO, VIANA, 2017].

Poly (ε-caprolactone) (PCL) has flexible properties and better mechanical strengths, being a candidate for grafting and stimulating cell regeneration [QIN, WU, 2012]. Gelatin, on the other hand, is widely used in tissue engineering and cell culture [GANG et al., 2018].

Based on this, the development of a PCL/gelatin scaffold for the future filling and repair of injured tissues was aimed.

2. METHODOLOGY

PCL (Aldrich 440744-250G, Mn 70000-90000) CAPA 6500 type - reported molar mass of 50,000 grams per molecule (g/mol) and Gelatin (Sigma-Aldrich, CAS Number: 9000-70-8 MDL: MFCD00081638) bovine skin - Type B were used.

Sample Preparation

In a chloroform flask (Vetec Chemistry), the PCL was dissolved under magnetic stirring (Fisatom - model 753A). After homogenization, the solution was placed in the rotary jet spinning chamber that allowed the flow of the polymeric solution [BRITO, 2013; RIGON, 2013; ZAVAGLIA et al., 2012]. After 24 hours and the solvent evaporation, the fibers were removed from the collector and stored in a desiccator before use. Gelatin was added and the sample crosslinked in glutaraldehyde for 24 hours. Care in washing material in distilled water has been repeated several times [GANG et al., 2018; LIU et al., 2015].

Characterization

Fiber morphology was observed in Zeiss Axio Vert A1 inverted light microscope (Zeiss). We evaluated by Fourier Transform Infrared Spectroscopy (FTIR) the functional groups and vibrational modes characteristic of each polymer in the Spotlight 400 FTIR equipment located at the Electronic and Optical Spectroscopy Laboratory. The parameters adopted were a measuring range from 4000 to 500cm^{-1}, assuming the Attenuated Total Reflectance (ATR) technique, with a resolution of 1cm^{-1} in 4 scans for each measurement.

Cell Culture

Vero cells were used, which is a cell line established from an African green monkey kidney (*Cercopithecus aeothiops*). These cells were cultivated in culture medium 199 (Lonza) with 10% Fetal Bovine Serum (FBS, Nutricell Cellular Nutrients, Campinas, SP, Brazil) at 37°C in an incubator with 5% CO_2. The medium changes occurred whenever it was acidified and the subcultures were done once or twice a week. Vero cells are recommended for studies on cytotoxicity and cell interactions in biomaterials [ISO 10993-5, 2009].

In vitro Direct Contact Toxicity

The samples (PCL, Gelatin and PCL/Gelatin) were placed in culture plates and kept in FBS-free medium for 24 hours in a 37°C incubator and 5% CO_2 oven. Then, Vero cells were inoculated at a concentration of 1.0 x 10^5 cells/ml in 24-well plates in 10% SFB medium. The cells were kept with the same culture parameters described above for 24 hours in direct contact with the studied materials. Later it was observed in Zeiss Axio Vert A1 inverted light microscope (Zeiss).

3. RESULTS and DISCUSSION

Fiber Characterization

Analyzing Figure 1, we verify the morphology of the samples. As qualitative characteristics, we can observe that PCL/Gelatin fibers were thicker and with smaller spacing compared to pure PCL. Gelatin took a non-porous form.

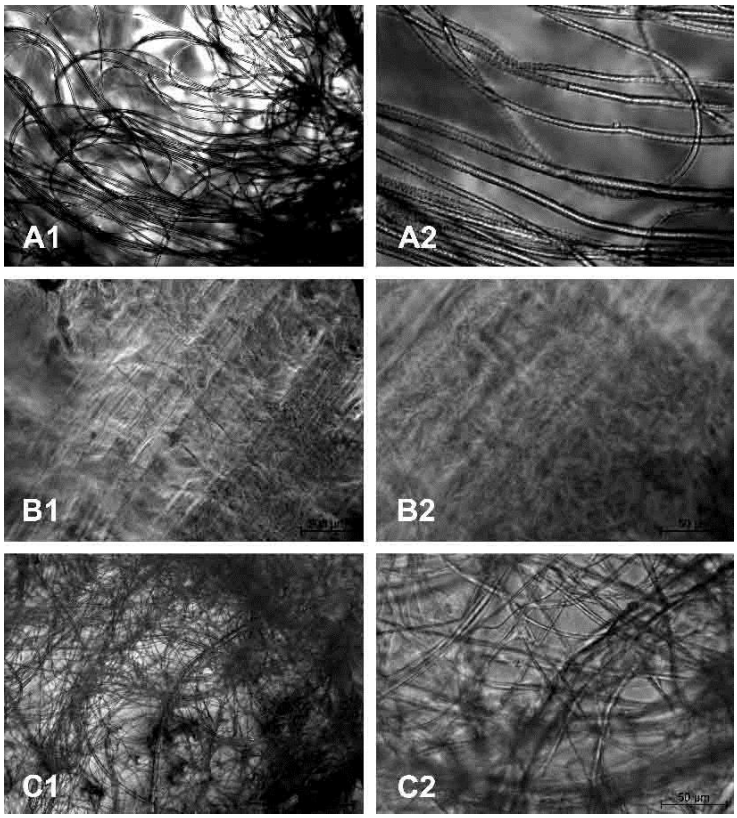

Figure 1. Light microscopy analysis of the materials studied. In figure A1 and A2 we verify the PCL in two increments 200 µm and 50 µm respectively. The same scale bars were used for corresponding Gelatin B1 and B2, C1 and C2 are PCL/Gelatin. Source: Prepared by the author.

For the FTIR, we observed the PCL with 2947 cm^{-1} vibrational mode, characteristic of the symmetrical CH_3 group, at 2867 cm^{-1} corresponds to the symmetrical CH_2. At 1471 cm^{-1} we have the asymmetric CH_3 stretch and at 1242 cm^{-1} we have the behavior of the complexes belonging to the COOH ethers. The peak of 2947 cm^{-1} is observed in the PCL, the vibration belongs to the asymmetric stretch of CH_2 (νCH2). We observed a narrow vibrational mode of 2867 cm^{-1} in the PCL, which belongs to the symmetrical stretch of CH_2 (νCH2) [VIDA et al., 2017]. These results can be seen in Figure 2.

In gelatin, we observed peaks of 1535 cm^{-1}, 1455 cm^{-1} [ZHUANG et al., 2015], 1242 cm^{-1} Amide III and collagen, 1080 cm^{-1} mode for collagen [MOVASAGHI et al., 2008]. This data can be seen in Figure 2.

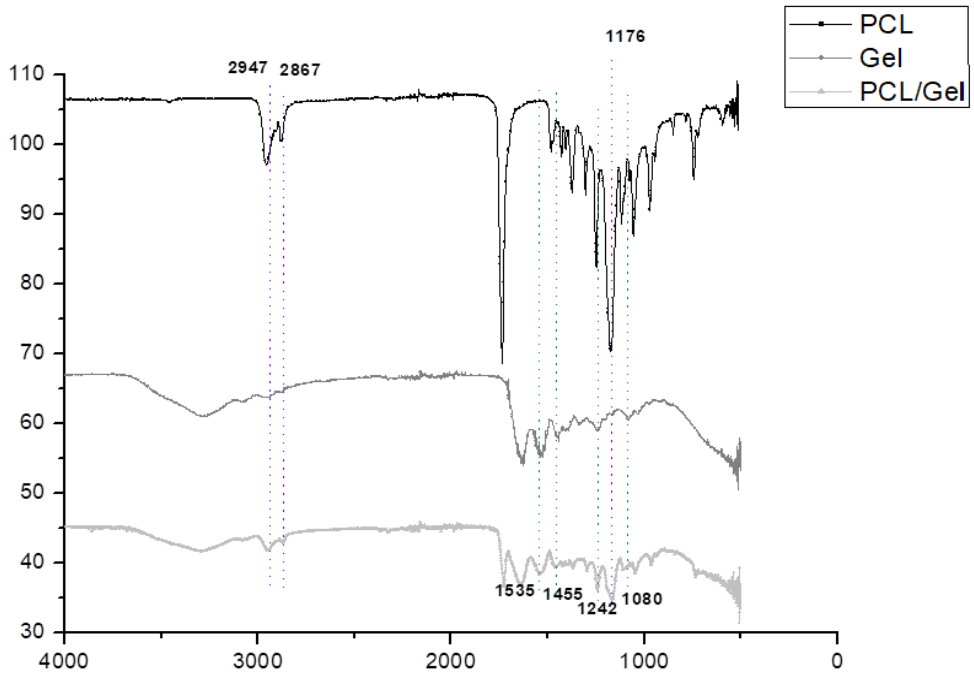

Figure 2. FTIR analysis of PCL, Gelatin and PCL/Gelatin fibers. Source: Prepared by the author.

In vitro direct contact toxicity

Figure 3 shows the 24 hours incubation direct contact cytotoxicity test data seen in phase contrast. Quantitatively, our data show no contact toxicity. We did not observe cellular changes promoted by the materials.

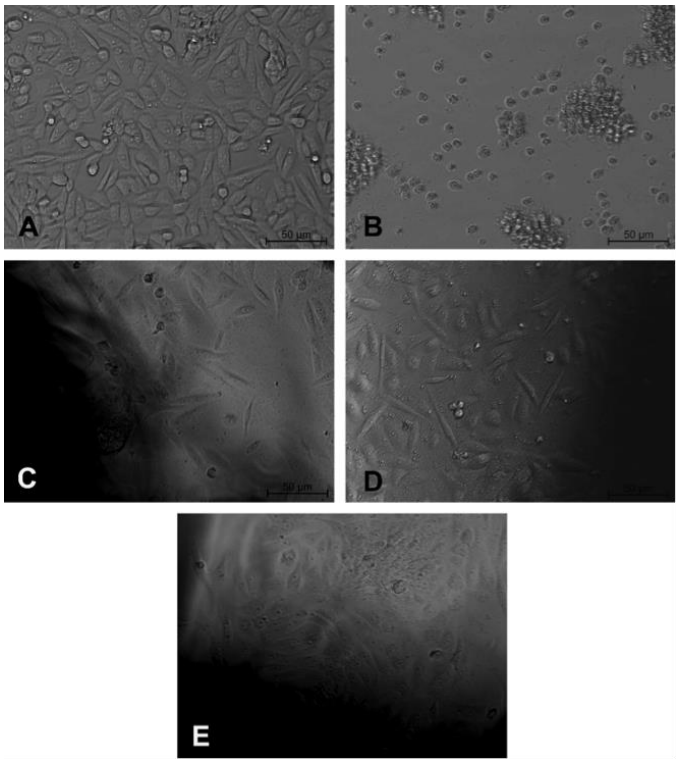

Figure 3. Contact toxicity analysis of cells observed in phase contrast with the different experimental groups with 24h incubation. A) Negative control of toxicity; B) Positive toxicity control; C) PCL; D) Gelatin; E) PCL/Gel. These figures do not show contact toxicity. Scale bar = 50 µm. Source: Prepared by the author.

These data are compatible with those presented by Vida et al. (2017) who worked with PCL, PLLA and PCL/PLLA fibers. As also LIU et al. (2015) referring to Gelatin.

When we have such a serious injury, as large scale burnings, soft tissues are damaged which could expose even bone component. Thinking about it we opted for PCL. But when PCL is jet spinning, difficult manipulation has been detected.

However, due to its bioresorbable characteristics, it would be classified as a temporary material. Inducing the individual's extracellular matrix (ECM) production guided by the material that would later be degraded, would aid tissue recovery.

Already non-crosslinked gelatin normally solubilized in the aqueous medium is easily eliminated from the body in vivo.

Our results suggest the use of PCL/Gelatin as a potential scaffold to improve mechanical properties and guide a repair environment over time.

Further research will be conducted accordingly.

CONCLUSION

Through the rotary jet spinning technique, it was possible to produce fibers. It was also possible to incorporate gelatin into the PCL fibers produced. The scaffolds were not toxic and are promising for tissue engineering applied to the skin.

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