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STEM CELL GROWTH ON DIFFERENT THICKNESSES OF PCL/GELATIN NANOFIBER SCAFFOLDS

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INTRODUCTION

Biodegradable nanofibrous scaffolds mimic the natural extracellular matrix, providing an ideal structure for cell growth. Polycaprolactone (PCL) and gelatin blends are biodegradable and provide a combination of good mechanical properties of PCL with gelatin's hydrophilicity and cell adhesion-promoting character [1]. Low-temperature hydrogen peroxide gas plasma (LTP) sterilization is an effective method for PCL scaffolds and enhances biocompatibility [2]. Mesenchymal stem cells have great therapeutic potential in regenerative medicine [3]. This work aimed to evaluate the influence of PCL/gelatin nanofibrous scaffold thickness on cell adhesion and growth of mesenchymal stem cells.

METHODOLOGY

- Materials: PCL, Mn = 80,000 g/mol from Sigma-Aldrich, gelatin (type B) from Thermo Fisher Scientific, and glacial acetic acid from Quimex (99.7%).
- Scaffolds with different thicknesses were prepared by electrospinning PCL/gelatin (2:1) in acetic acid for different durations (A = 1 h, B = 1.5 h, C = 2 h, and D = 3 h) under the same conditions (1 mL/h, 15 kV, 12 cm). The scaffolds were sterilized with LTP and characterized before and after sterilization by scanning electron microscopy (SEM) and Fourier transform infrared spectroscopy (FTIR). Biological tests were carried out on rabbit mesenchymal stem cells. Cytotoxicity tests were executed using flow cytometry with FITC Annexin V staining, adhesion and viability tests were accomplished by seeding stem cells on each sterilized scaffold (1.0×1.0 cm2) with different thicknesses, incubated in non-adherent 24-well plates. The cells were counted using the Neubauer chamber (1, 2 days) and by fluorescence of resazurin metabolization (1, 2, 3, 4, 7, 10 days).

RESULTS AND DISCUSSION

- SEM: scaffolds retained their characteristic fibers after sterilization, however, there were melting points at the intersection between the fibers, and some fibers showed a melted appearance.
- FTIR: characteristic bands remained the same before and after sterilization and between different thicknesses. Bands' intensity increased with increasing thickness, suggesting that LTP does not alter the chemical composition of PCL/gelatin structures.
- Cytotoxicity: scaffolds are not cytotoxic, and the thickness does not interfere with the apoptosis and cell necrosis results.
- Cell adhesion and viability tests: counting cells with Neubauer chamber, the number of cells decreased for the thicker scaffold D when comparing to scaffolds A and B, indicate some difficulty in cell adhesion and growth until 2 days due to the morphology of scaffold D, which had smaller diameter fibers and smaller pores between them [4]. In the fluorescence test, all the scaffolds showed a constant number of cells up to 3 days. Thicker scaffolds (C and D) presented fewer cells than the thinner ones (A and B) in the first days, possibly due to their smaller pores [4], however the opposite was observed on day-10. Also, scaffold D showed the least variation in the number of cells over time, indicating better stability in cell maintenance during the study period, which may also be linked to a possible increase in pores over time due to the solubilization of gelatine.

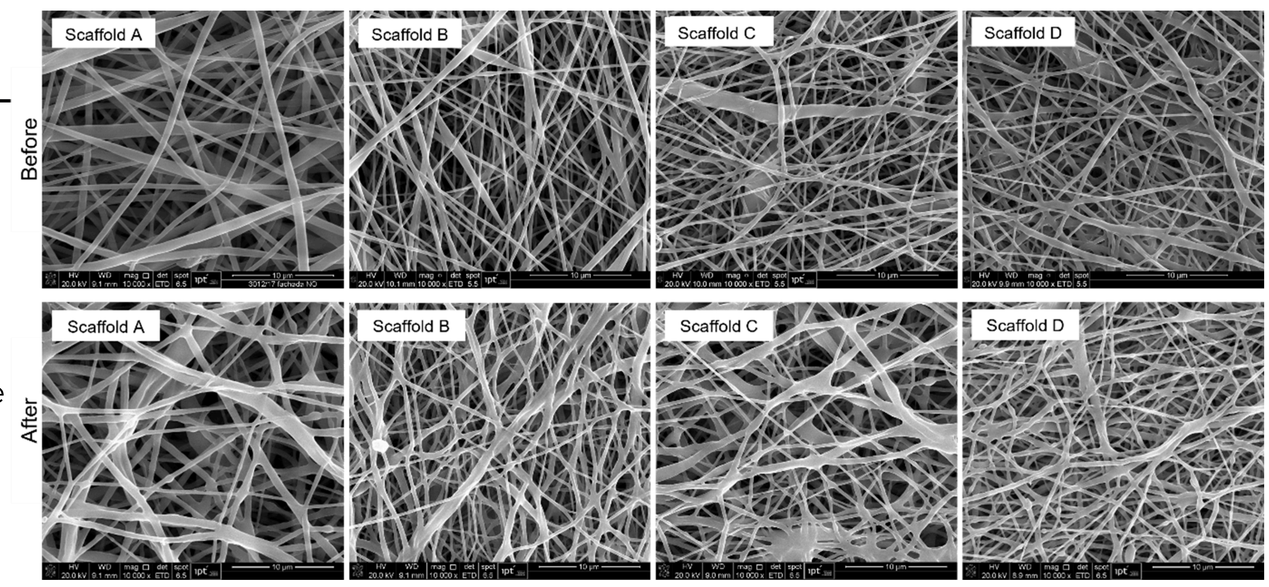

Figure 1 – SEM images of PCL/gelatin scaffolds before and after LTP sterilization, magnification of 10000x.

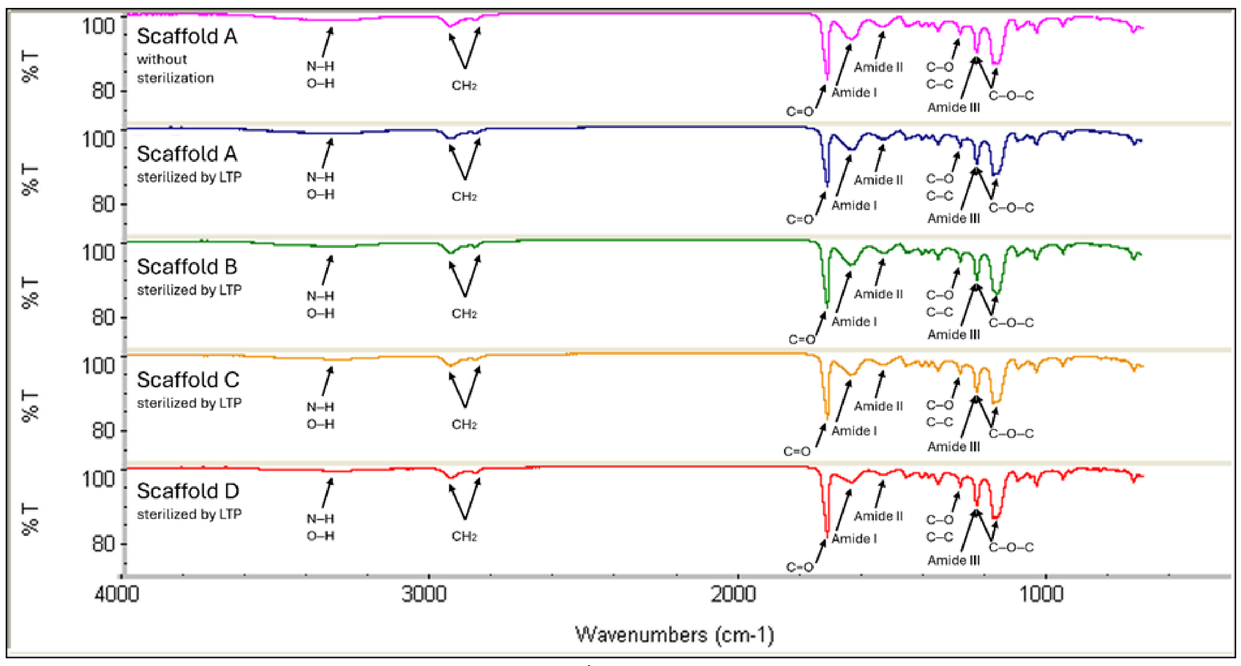

Figure 2 – FTIR spectra of PCL/gelatin scaffolds without sterilization treatment (scaffold A) and sterilized by LTP for the four different thicknesses (A, B, C, and D). (%T = % Transmittance)

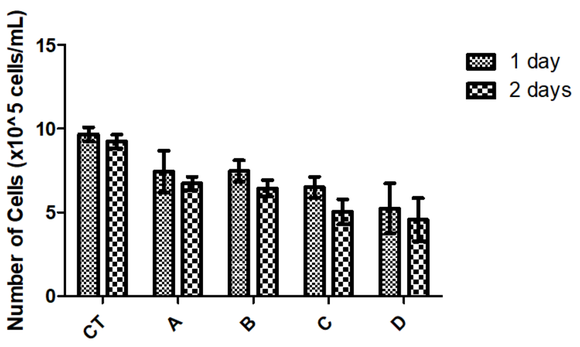

Figure 3 – Number of cells in each sample counted using a Neubauer chamber.

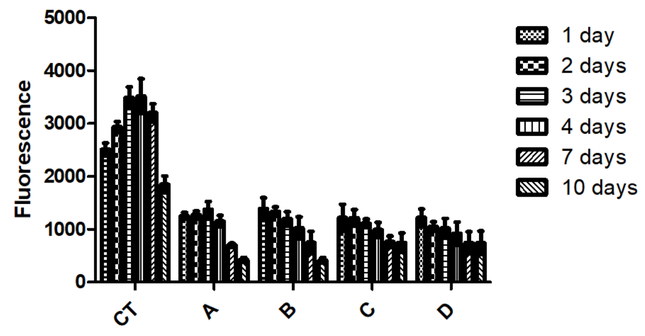

Figure 4 – Number of cells in each sample counted indirectly by fluorescence of resazurin metabolization in a plate reader.

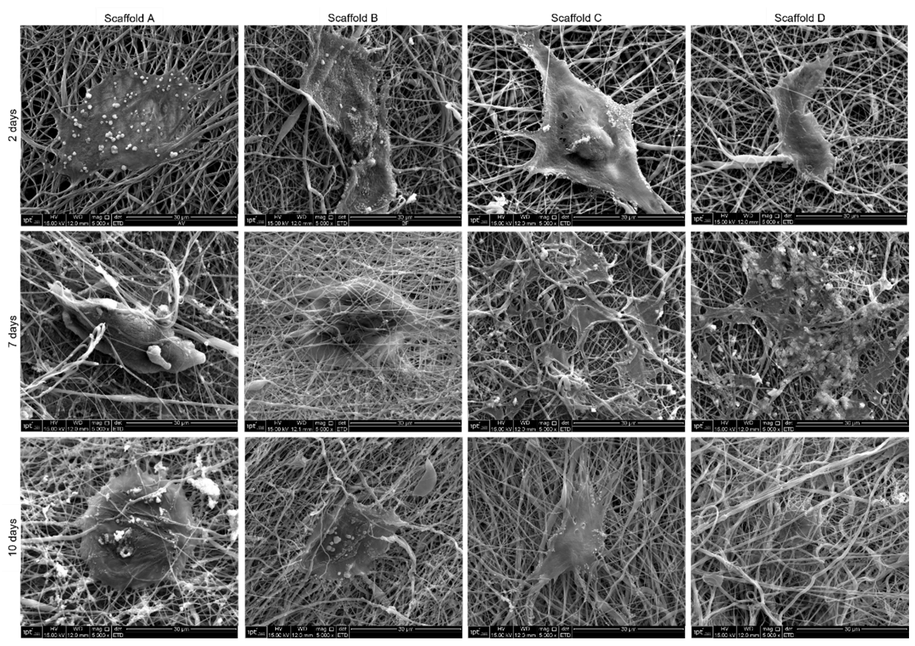

Figure 5 – SEM images of cells on scaffolds of different thicknesses at 5000x magnification.

CONCLUSION

LTP sterilization is a suitable method for sterilizing PCL/gelatin scaffolds since it does not change the chemical composition of the scaffolds. The cytotoxicity tests indicate that they are not cytotoxic, and the thickness does not interfere with the apoptosis and cell necrosis results. Initially, thinner scaffolds present better results in cell viability tests for mesenchymal stem cells until 3 to 4 days, possibly due to their larger pores, while the thickest one shows better stability in maintaining cells over time. These results indicate the importance of scaffold thickness depending on the biomedical application.

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