ELECTROSPUN MEMBRANES WITH ENHANCED BIOACTIVITY FOR PERIODONTAL TISSUE ENGINEERING APPLICATIONS

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Introduction

Periodontal disease affects the tooth supporting structures, namely alveolar bone, cementum and periodontal ligament. Current regenerative treatments show limited and unpredictable results, and fail to effectively promote a complete regeneration of the periodontal tissues damaged or lost due to the disease [1]. Therefore, new alternative strategies are needed to improve clinical outcomes. Decellularized cell-derived extracellular matrix (dECM) offers the opportunity to enhance the bioactivity of the scaffolds, and has been explored for tissue engineering (TE) applications since it can mimic the in vivo microenvironment [2].

In this work, electrospun polycaprolactone/chitosan (PCL/CTS) nanofibrous membranes loaded with dECM were developed using lyophilized dECM powders derived from human Periodontal Ligament Stem Cells (PDLSCs) incorporated in the electrospinning polymeric solutions.

Methods

Following a previously established protocol, PDLSCs were cultured for 10 days and decellularized to obtain dECM, which was then lyophilized [3]. PCL, PCL-CTS and PCL-CTS-ECM membranes were fabricated by electrospinning using the following parameters, needle gauge: 21G; flow rate: 0.5 mL/h; voltage: 24 kV; needle to collector distance: 22 cm; temperature: 23-24 °C; relative humidity: 30-40%. The morphology of the electrospun fibers was characterized by scanning electron microscopy (SEM). The mechanical properties of the membranes were assessed through uniaxial tensile testing using a mechanical tester (UniVert Model UV-200-01, CellScale). PDLSCs were seeded on PCL, PCL-CTS and PCL-CTS-ECM membranes and cultured in osteogenic medium for 21 days. Alkaline phosphatase (ALP) and collagen I (COL I) gene expression was determined by quantitative real-time Polymerase Chain Reaction (qRT-PCR) analysis.

Results

The electrospun membranes were composed of beadless and homogeneous nanofibers, showing high porosity and interconnectivity (Figure 1A-C). PCL-CTS and PCL-CTS-ECM membranes showed similar mechanical properties, which were decreased compared to PCL (Figure 1D). PCL-CTS-ECM membranes significantly promoted cell proliferation in comparison to the other membranes (Figure 1E). *ALP* and *COL I* gene expression exhibited a statistically significant increase in PCL-CTS-ECM membranes (Figure 1F).

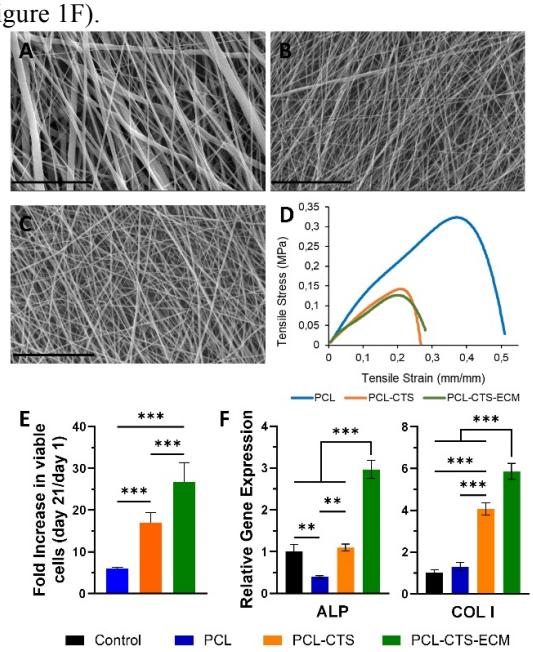

*Figure 1: SEM images of PCL (A), PCL-CTS (B), and PCL-CTS-ECM (C) nanofibers. Scale bar: 8 µm. Representative stress-strain curves (D). Fold increase (E) in the number of viable cells on day 21 (in relation to day 1). F: ALP and COL I gene expression by PDLSCs after 21 days of osteogenic differentiation on the electrospun membranes. Values are expressed as mean ± SD (N = 3).** $p < 0.01$ and *** $p < 0.001$.*

Discussion

Our results show that dECM incorporation did not affect the membrane's properties, as loaded membranes maintained similar fiber morphology and mechanical properties of PCL-CTS membranes. PCL-CTS-ECM membranes promoted cell proliferation and enhanced the osteogenic differentiation of PDLSCs, confirmed by increased *ALP* and *COL I* gene expression, in comparison to PCL and PCL-CTS membranes, due to the presence of dECM. This work describes the use of lyophilized dECM to yield electrospun membranes with enhanced bioactivity for periodontal (TE) applications.

References

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3. Santos et al, ACS Appl Nano Mater, 7: 4501–4517, 2024