Doctoral Dissertation
Doctoral Program in Bioengineering and Medical-Surgical Science (37th Cycle)

Synergistic combination of stem cell-based approach and automated controlled dynamic culture for advanced dental tissue engineering

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Summary

The periodontal ligament (PDL) is a highly specialized connective tissue that plays a crucial role in maintaining periodontal homeostasis and supporting tooth function. Composed of a hierarchically organized collagen fiber network and a heterogeneous population of cells, including periodontal ligament stem cells (PDLSCs), the PDL exhibits remarkable regenerative potential. However, when affected by periodontitis—a prevalent degenerative disease—its functionality deteriorates, leading to alveolar bone resorption and eventual tooth loss. Conventional treatments focus on mechanical debridement and inflammation control but have often yielded limited regenerative outcomes. To address this challenge, periodontal tissue engineering has emerged as a promising field, leveraging biomimetic scaffolds and mechanical stimulation to enhance the regenerative capability of PDLSCs.

This thesis explores the effects of controlled mechanical stimuli—specifically stretch and shear stress—on human-derived PDLSCs (hPDLSCs) in biomimetic environments. Given that mechanical loading is a key regulator of PDL function, understanding how PDLSCs respond to specific mechanical cues is essential for optimizing tissue engineering strategies. In particular, the thesis involved the development of three experimental platforms: (i) polydimethylsiloxane (PDMS) flexible substrates combined with a stretch bioreactor, (ii) three-dimensional (3D) biomimetic electrospun polycaprolactone (PCL) scaffolds combined with a stretch bioreactor, and (iii) three-dimensional (3D) thermoplastic polyurethane (TPU) scaffolds integrated within a perfusion bioreactor.

For the first platform, a customized flexible PDMS substrate was developed through an iterative process of design, modeling, manufacturing, and mechanical characterization. Finite element (FE) and digital image correlation (DIC) analyses were used to evaluate strain distribution at the bottom of the substrate, providing insight into the effective stimulations experienced by adherent cells. The system was validated through *in vitro* biological experiments in which hPDLSCs were subjected to different stretching protocols, demonstrating that the timing of stretch stimulation is crucial for inducing hPDLSCs alignment and upregulating both osteogenic and PDL-related gene expressions, highlighting possible osteogenic and ligamentogenics potential of hPDLSCs.

Building upon this approach, in the second stretch platform the biomimicry was improved by integrating electrospun PCL scaffolds. Different scaffold formulations were developed and characterized in terms of mechanical and morphological properties, allowing for the selection of the most suitable variant for biological applications. The optimized PCL scaffold was combined with the stretch bioreactor. Biological experiments, performed with hPDLSCs, confirmed that stretch stimulation induced cell alignment and demonstrated that the biomimetic scaffold, when combined with stretch stimulation, led to the overexpression of PDL-related genes, further supporting the role of mechanical stimulation and biomimetic scaffolds in directing cellular behavior.

In parallel, the perfusion platform was developed to explore the effects of fluid-induced shear stress on hPDLSCs, a mechanical cue that has been poorly studied *in vitro*. TPU scaffolds with varying pore sizes were 3D-printed. To support the identification of the most suitable scaffold, they were characterized in terms of morphology and permeability, and cellular

viability and proliferation were evaluated under static conditions. Moreover, wall shear stress distribution on the scaffold filaments was assessed via computational fluid dynamics (CFD) simulations. The optimal scaffold was integrated with a previously developed perfusion bioreactor, and biological experiments were carried out with hPDLSCs. Gene and protein expression analyses indicated that shear stress modulates cellular behavior, leading to significant overexpression of key genes and the deposition of proteins, providing new insights into its role in PDL regeneration.

Collectively, this work underscores the significance of biomimetic scaffolds and mechanical stimulation in PDL tissue engineering. By characterizing and optimizing mechanical cues, this study contributes to the development of more effective regenerative strategies for periodontal diseases, paving the way for improved therapeutic approaches.