PERFUSION DRIVEN SHEAR STRESS REGULATES PERIODONTAL LIGAMENT STEM CELL BEHAVIOR IN 3D PRINTED SCAFFOLDS

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Introduction

In vivo, the periodontal ligament (PDL) is exposed to mechanical forces such as compression, tension, and shear stress, which are known to play a crucial role in regulating cell growth, cell differentiation, and tissue development [1]. *In vitro*, the effect of shear stress on PDL cells remains to be elucidated. In this study, we investigated the impact of fluid-induced shear stress on human PDL stem cells (hPDLSCs) seeded into three-dimensional (3D) thermoplastic polyurethane (TPU) scaffolds and cultured within a direct perfusion bioreactor.

Materials and Methods

TPU scaffolds (diameter = 10 mm; height = 5 mm) with varying pore sizes (100, 200, and 300 µm) were designed and fabricated using a 3D printer (i3 mk3s+, Prusa Research). Computational fluid dynamics (CFD) simulations (COMSOL Multiphysics 6.0, Comsol Inc.) were conducted for each scaffold geometry, imposing a flow rate of 0.3 mL/min, to estimate the fluid-induced shear stress developing on the scaffold structure. To assess the optimal scaffold design, preliminary biological experiments were performed. The scaffolds were seeded with hPDLSCs (1.5×10^6 cells per scaffold) and the constructs were cultured under static conditions for 10 days, with Alamar Blue assays conducted on days 1, 4, 7, and 10. Once defined the optimal scaffold geometry (pore size = 200 µm), the hPDLSCs-seeded constructs were pre-cultured under static conditions for 7 days, followed by additional 7 days of direct perfusion culture in a bioreactor (Fig. 1A) [2] at a flow rate of 0.3 mL/min (n = 3). Control constructs were maintained under static conditions for the entire duration. Alamar Blue assays were performed on days 1, 7, and 14. Confocal microscopy and quantitative real-time PCR (qRT-PCR) analyses were conducted at day 14.

Results

CFD simulations revealed fluid-induced shear stress values of 5.42, 2.40, and 1.43 mPa for scaffolds with pore sizes of 100 µm, 200 µm (Fig. 1B), and 300 µm, respectively. From the cellular viability tests at day 10 (8000 a.u, 12000 a.u., 11000 a.u. for 100 µm, 200 µm, and 300 µm pore-size scaffolds, respectively), the 200 µm pore-size scaffold was selected as the optimal geometry. The Alamar Blue assays showed that perfusion did not affect cellular metabolic activity, although confocal analysis revealed a reduced number of cells in perfused constructs (Fig. 1C). Furthermore, in constructs cultured under perfusion, the qRT-PCR analysis showed increased expression of collagen type I and osteopontin, typical of tooth root development [3], and PDL-related gene collagen type XII, responsible for inhibiting the tissue mineralization [4].

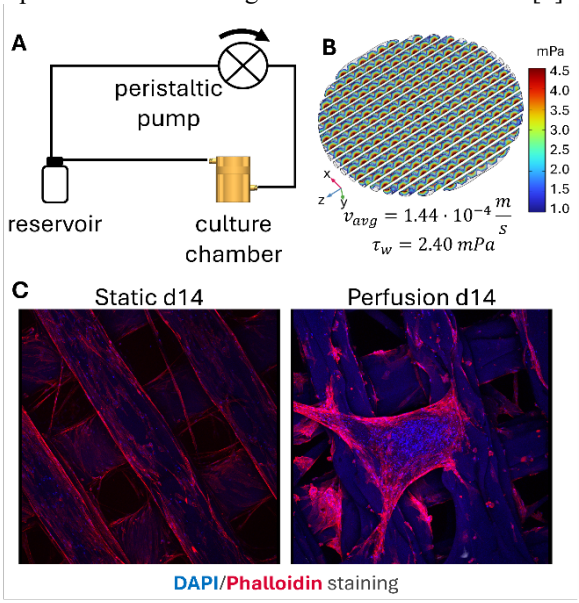


Figure 1. (A) Perfusion bioreactor scheme; (B) CFD results for the 200 µm pore-size scaffold, only a layer is represented; (C) Dapi/Phalloidin staining performed at day 14 on static (left) and perfused (right) scaffold (cell nuclei in blue, actin filament in red).

Conclusions

In this study, we demonstrated that fluid-induced shear stress influences the mechano-biological behavior of hPDLSCs cultured on TPU scaffolds. Notably, direct perfusion promoted the upregulation of markers associated with extracellular matrix synthesis and PDL-specific genes, suggesting the potential multilineage differentiation of hPDLSCs. These results offer important insights for the development of novel PDL tissue engineering strategies and regenerative medicine applications. Further biological experiments are ongoing to investigate the signaling pathways triggered by fluid-induced shear stress in hPDLSCs.

References

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