IN VITRO PLATFORM TO STUDY BONE MECHANOBIOLOGY UNDER FLUID-INDUCED SHEAR STRESS AND INTERMITTENT PRESSURE

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Introduction

The musculoskeletal system is continuously exposed to physical loads. At the microscale, bone cells are subjected to interstitial fluid-induced shear stress (~0.5–24 mPa [1]) and unsteady pressure (rest: ~3–8 kPa; activity: ~10–35 kPa [2]), which play a crucial role in regulating bone remodelling. *In vitro,* fluid-induced shear stress and hydrostatic pressure were shown to enhance proliferation and osteogenic differentiation of mesenchymal stem cells [1]. However, further research is needed to elucidate the bone mechanobiology associated with each stimulus and their combinations. Here, we developed an *in vitro* research platform based on three-dimensional (3D) biomimetic bone tissue models and an automated bioreactor delivering, for the first time, combinable bone-like shear stress and intermittent pressure.

Methods

The bioreactor is composed of a culture chamber (CC) inserted in a closed-loop perfusion unit [3] equipped with a controlled pinch valve downstream the CC (Fig. 1A). The perfusion unit generates fluid-induced shear stress through the cultured construct and, when the pinch valve cyclically closes, subjects the construct to intermittent pressure (closing period $T = 0.25$-30 s; duty cycle $DC = 1$-100%). Placing a pressure sensor between the CC and the pinch valve, the pressure values reached within the CC were characterized varying the imposed T and DC. The biomimetic bone tissue model is based on a 3D-printed poly-lactic acid scaffold (diameter = 10 mm; height = 5 mm) seeded with human bone marrow-derived mesenchymal stem cells (BM-MSCs) mixed with 150 µL of collagen (2×10^6 cells/scaffold). For preliminary biological tests, after 1 day of static culture, the construct was housed in the CC and exposed for further 14 days to direct perfusion (DP, flow rate = 0.3 mL/min) without or with intermittent pressure (IP, T = 10 s, DC = 70%, 2 h/day for mimicking a physiological daily loading) in incubator (37°C, 5% CO_2) and controls were cultured statically (for each condition, n = 3). Cell viability was evaluated by resazurin assay, osteogenic gene expression was analyzed by RT-qPCR.

Results

Characterization tests confirmed the performances of the pinch valve, with measured mean peak pressure values in the bone-like pressure range (4.96-24.63 kPa). Imposing $T = 1$ s and $DC = 70\%$, the peak pressure range (3.43-6.01 kPa) was comparable to that typical for the resting condition, while $T > 10$ s and $DC = 70\%$ led to activity-like pressure values (8.20-29.82 kPa, Fig. 1B). Preliminary biological tests demonstrated enhanced cell viability (Fig. 1C) and an increasing trend of osteogenic gene expression (Fig. 1D) under combined stimuli compared to direct perfusion only.

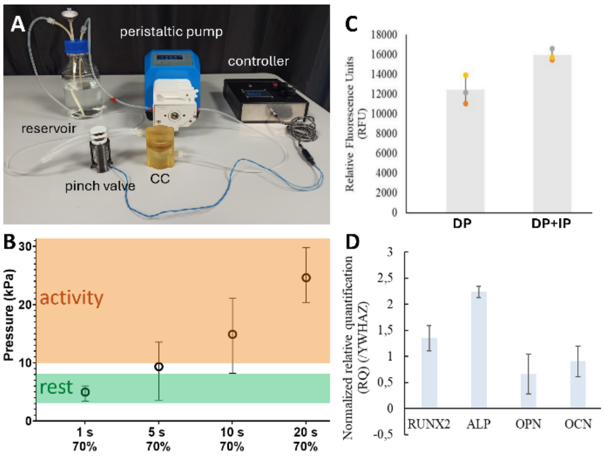

Figure 1: A) Bioreactor set-up; B) Measured peak pressure values; C) BM-MSCs metabolic activity; D) Osteogenic gene expression analysis of BM-MSCs cultured under DP+IP in comparison to DP, bars represent the mean fold change of 3 replicates +/− standard error of the mean.

Discussion

The proposed research platform enables investigating *in vitro* the mechanobiological response of bone tissue to its most relevant physical stimuli, i.e., fluid-induced shear stress and intermittent pressure. Preliminary tests showed that their combination could promote cell viability and osteogenic differentiation. Further tests are ongoing to explore the effects of stimulations applied for longer periods and/or with higher amplitudes.

References

1. Yeatts & Fisher, Bone 48, 171–181, 2011.
2. Gurkan et al., Ann Biomed Eng 36:1978–1991, 2008.
3. Gabetti et al., Sci. Rep. 12:13859, 2022.

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