Direct perfusion and intermittent pressure bioreactor for mimicking and investigating the bone environment

DIRECT PERFUSION AND INTERMITTENT PRESSURE BIOREACTOR FOR MIMICKING AND INVESTIGATING THE BONE ENVIRONMENT

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Introduction

In physiological conditions, the musculoskeletal system is exposed continuously to physical loads. As to bone microstructure, cells are subjected to interstitial fluid flow-induced shear stress (~0.5-24 mPa [1]) and unsteady pressure (rest: ~3-8 kPa, activity: ~10-35 kPa [2]), strongly involved in regulating bone remodeling. *In vitro*, fluid shear stress and hydrostatic pressure were demonstrated to enhance mesenchymal stem cell proliferation and osteogenic differentiation [1]. Still, for future tissue engineering and precision therapies, further research is needed to unravel the contribution of each stimulus and of their combination on bone tissue. To this end, a previously developed investigation platform [3,4], based on three-dimensional (3D) biomimetic bone tissue models and an automated bioreactor providing direct perfusion, has been upgraded for delivering, for the first time, *in vitro* native-like combined shear stress and intermittent pressure and for studying in-depth the induced bone tissue response.

Methods

The bioreactor (Fig. 1A), consisting of a 3D-printed culture chamber (CC) inserted in a closed-loop direct perfusion unit (DPU) [3], has been equipped with an intermittent pressure unit (IPU). A controlled pinch valve (Cole Parmer) has been located downstream of the CC for inducing intermittent pressure waveforms by cyclically closing. An Arduino-based controller allows setting the pinch valve closing period (T=0.25-30 s) and duty cycle (DC=1-100%). To test and characterize the IPU, biomimetic poly-lactic acid scaffolds [5] loaded with 200 µL of collagen (Merck) were housed in the CC and exposed to a defined flow rate (Q=0.3 mL/min) and different operating conditions (T=10-30 s, DC=50-70%), while pressure values were measured with a non-invasive sensor (HJK) placed between the CC and the pinch valve. For preliminary biological tests, human mesenchymal stem cells mixed with 200 µL of collagen were poured onto the scaffolds (3×10^6 cells/scaffold) and, after 1 day of static culture, the bone tissue models (n=2) were placed in the CC and exposed for further 6 days to direct perfusion (DP, Q=0.3 mL/min) with or without intermittent pressure (IP, T=10 s, DC=50%) in incubator (37°C, 5% CO_2), while controls were cultured statically (n=2). At day 7, cell viability was evaluated by live/dead staining and Resazurin reduction assay.

Results

Characterization tests confirmed the IPU proper functioning, with mean peak pressure values in the range of the native pressure values (5.27-15.09 kPa): imposing T = 10 s and DC = 50%, the peak pressure range (2.25-7.84 kPa) was comparable to that typical for the resting condition; while T = 30 s and DC = 70% led to activity-like pressure values (12.83-17.60 kPa, Fig. 1B). Live/dead staining showed higher cell viability for constructs cultured within the bioreactor with respect to static culture, with moderately higher viability when IP was added (Fig. 1C, D).

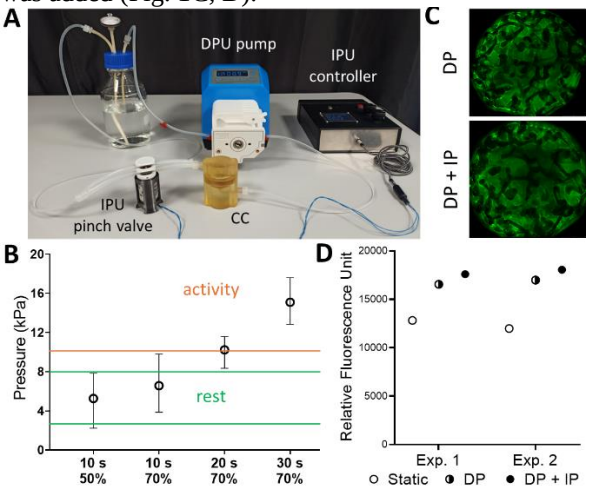

Figure 1: A. Bioreactor set-up; B. Measured peak pressure values; C. Live/dead staining of bone tissue models cultured under direct perfusion (DP) without or with intermittent pressure (IP); D. Resazurin reduction assay.

Discussion

The upgraded bioreactor allows culturing 3D bone tissue models under native-like conditions, combining for the first instance shear stress and intermittent pressure, promoting cell viability. Similarly to a previous study, where the signalling pathways activated by culture under DP and pulsed electro-magnetic field conditions were unravelled by RNA-Seq analysis [4], in the next future additional experiments and advanced biological analyses will be performed for uncovering the multi-factorial impact of the imposed physical stimuli on bone cells and tissue.

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.
4. Daou et al., Bone 182:117065, 2024.
5. Zenobi et al., Bioengineering 10(5), 567, 2023.

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