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# 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  $\mu\text{L}$  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  $\mu\text{L}$  of collagen were poured onto the scaffolds ( $3 \times 10^6$  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 ( $37^\circ\text{C}$ , 5%  $\text{CO}_2$ ), 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).

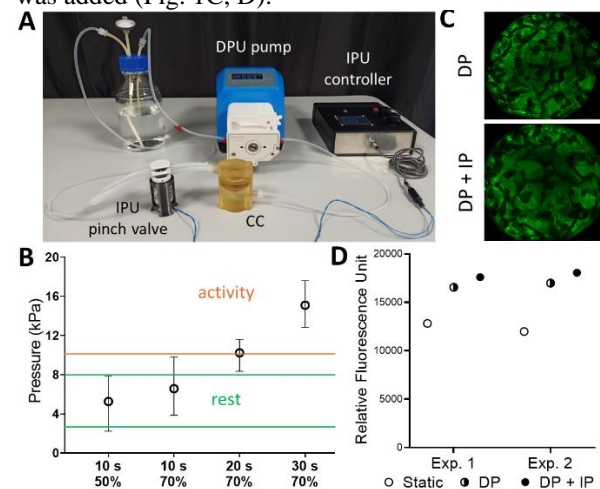


Figure 1: A. Bioreactor set-up; B. Measured peak pressure values; C. Live/dead staining of bone tissue models cultured under direct perfusion (DP) without or with intermittent pressure (IP); D. Resazurin reduction assay.

## 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

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