AUTOMATED AND PARALLELIZED MULTI-STIMULI BIOREACTOR FOR BONE MECHANOBIOLOGY INVESTIGATIONS

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Introduction

Bone regeneration *in vivo* is governed by the interplay of mechanical stimuli. At the cellular scale, intraosseous hydrostatic pressure (HP, daily activity: ~3–8 kPa, intense activity: ~10–35 kPa [1]) and interstitial fluid flow–induced shear stress (FSS, ~20-5000 mPa [2]) represent key cues regulating cell behaviour. While the individual effects of these stimuli *in vitro* are well proven, their combined action in three-dimensional (3D) bone tissue models remains poorly understood due to the lack of suitable experimental platforms. In this view, we developed an automated parallelized bioreactor providing, for the first time, tunable combinations of FSS and intermittent HP to 3D bone tissue models, and we investigated the effects of the combined stimuli on the early biological response of 3D bone tissue models.

Methods

A previously developed direct perfusion bioreactor [3] was upgraded for delivering combinations of FSS and intermittent HP. The bioreactor comprises three culture chambers (CCs), integrated in three independent hydraulic circuits. Each intermittent HP unit consists in a pinch valve, placed downstream of the CC, and of a custom controller (Fig. 1A). Direct perfusion generates FSS in the cultured construct and, when the pinch valve cyclically closes, it subjects the construct to intermittent HP (closing period T = 0.25-30 s; duty cycle DC = 1-100%). Pressure values were characterized using a sensor (HJK) placed between the CC and the pinch valve, housing biomimetic scaffolds [4] loaded with collagen, imposing a set flow rate (Q = 0.3 mL/min) and varying T and DC. For biological tests, human bone marrow-derived mesenchymal stem cells (BM-MSCs) mixed in 200 µL of collagen were seeded into the scaffolds (2.5×10^6 cells/scaffold) and, after 1 day of static culture, the bone tissue models (n = 3) were placed in the CC and exposed for 7 days to direct perfusion without (DP) or with (DP+IP) intermittent HP (T = 10 s, DC = 50%) in incubator (37°C, 5% CO2, Fig. 1B). Osteogenic gene expression was analyzed by RT-qPCR.

Results

Characterization tests confirmed the intermittent HP unit performances, with measured mean peak pressures in the bone-like range (3.38-15.09 kPa, Fig. 1C). Particularly, imposing T = 10 s and DC = 50%, the peak pressure range (2.25-7.84 kPa) was comparable to that typical for *in vivo* daily activity, while T > 10 s and DC = 70% led to intense activity-like values (8.35-17.59 kPa, Fig. 1C). Constructs exposed to combined FSS and intermittent HP mimicking daily activity (DP+IP, mean peak pressure = 5.27 kPa) showed increased ALP expression (2.7-fold, Fig. 1D) and modest OPN upregulation (~1-fold) compared to constructs cultured under DP condition.

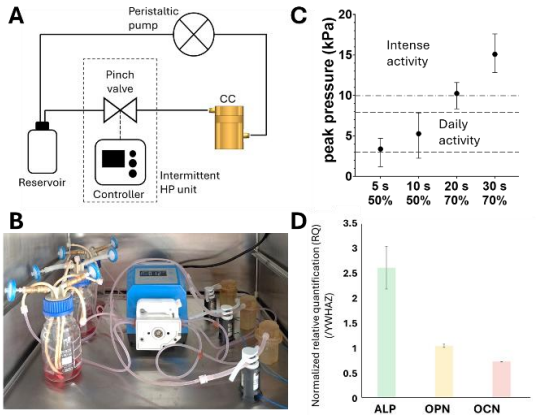


Figure 1: A. Bioreactor schematic; B) Measured peak pressure values; C. Parallelized bioreactor within the incubator; D. Osteogenic gene expression of BM-MSCs cultured under DP+IP in comparison to DP (mean fold change of 3 replicates +/− standard error of the mean).

Discussion

The proposed multi-stimuli bioreactor enables, for the first time, the concurrent delivery of tunable, physiologically relevant FSS and intermittent HP to 3D bone tissue models. Biological results showed that combined stimulation within daily activity–related ranges promoted early osteogenic differentiation. The bioreactor modular design will enable integration of additional physical cues, such as PEMF stimulation [5], and the tunability will support the development of advanced stimulation strategies. Overall, this platform represents a robust research tool for bone mechanobiology investigation.

References

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Acknowledgements

This study was carried out within the BIGMECH project – funded by European Union – Next Generation EU within the PRIN 2022 program (D.D. 104 - 02/02/2022 Ministero dell'Università e della Ricerca).