MECHANICAL LOADING OF *EX VIVO* BOVINE TRABECULAR BONE IN 3D-PRINTED BIOREACTORS

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Introduction

In 2021, 18.3% of the world's population was diagnosed with osteoporosis [1]. Bone biomechanical behaviour studies have shown that better understanding of bone tissue mechanics are key in the development of more effective strategies to prevent bone fracture, regenerate, and repair osteoporotic bone defects [2]. Although polycarbonate (PC) bioreactors have been successfully used to study tissue mechanics in bone cores *ex vivo* [3], they are difficult and expensive to fabricate, and the height-diameter ratio for bone cores is below the standard for mechanical compression tests [4]. A bioreactor system was developed using polyjet 3D printing and MED610™, which has been suggested as suitable for long-term culture [5]. This system had been validated in a previous study [6], however, leakage and other experiment limitations were observed. Thus, the objective of this study was to improve and continue the validation of the new bioreactor system in a long-term *ex vivo* bovine trabecular bone core experiment.

Methods

Twenty-two viable trabecular bone cores (10 mm height, 10 mm diameter) retrieved from a 3-year-old bovine sternum were individually cultured in custom-made 3D-printable MED610™ bioreactor chambers at 37°C and 5% CO_2, with constant culture medium perfusion (6.6 mL/hr), for 21-days (Figure 1a). Bone cores in bioreactors were ranked and sorted evenly based on their apparent elastic modulus (E_{app}) into control and loading groups (n = 11/group). The load group was stimulated mechanically (Figure 1b) five times per week with a "trapezoid" waveform that mimicked a physiological high-impact stimulus, with a Δ3000 με displacement and a strain rate of 3000 με/s for 120 cycles. Bone core E_{app} was assessed on days 0 and 21 in random order at room temperature with an initial 10 N pre-load, followed by a quasi-static -3000 με strain at -50 με/s. Data were analyzed in MATLAB 2020b (MathWorks) and E_{app} was calculated assuming linear elastic behavior with the last 50% of the linear force-displacement curve. A two-way ANOVA and a Tukey post-hoc tests ($\alpha = 0.05$) were used to compare statistical differences between groups on days 0 and 21. A two-tailed t-test was used to compare between groups for percent differences in E_{app} after the 21-days ($\%\Delta E_{app}$). Shapiro-Wilk tests confirmed normality across all groups. All statistical analysis were performed in SPSS® Statistics 23 (IBM) and GraphPad Prism® 6.1 (Dotmatics).

Results

Bone cores tested on day 0 had a mean and a standard deviation E_{app} of 97.7 ± 30.3 MPa, with no significant difference between groups after sorting. After 21-days, the E_{app} decreased in all bone cores by 22.4% (± 20.6% SD) on average (Figure 1c). No significant differences in neither E_{app} nor $\%\Delta E_{app}$ were observed between timepoints nor groups. Though not statistically different, there was a low % difference on day 0 between the E_{app} means of the load and control groups (0.1%), which increased to 7.3% after 21-days. Thus, the load group's E_{app} decreased less, on average, than the control group (19.8% ± 19.6% decrease versus 25.0% ± 22.3%).

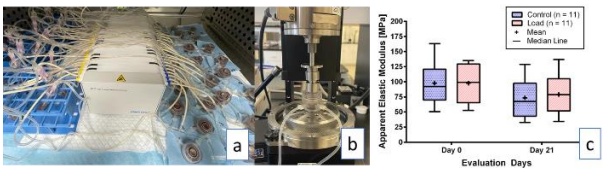

Figure 1 (a) 3D-printed MED610™ bioreactors containing bone cores setup with peristaltic pump, tubing, and sample specific medium reservoir; (b) Ball-and-socket fixture in Mach-1 System (Biomomentum) for bone cores mechanical compression testing and stimulation; (c) Apparent elastic moduli for both control and load groups in days 0 and 21.

Discussion

The 21-days experiment was successful: no system leakage nor infections were observed. And although data analyses results do not follow the same trends in E_{app} changes as other *ex vivo* trabecular bone core studies using bioreactors [3,6], it is still relevant that the $\%\Delta E_{app}$ decrease was higher in the control group. It is thought, then, that the mechanical stimulation might have helped in the overall bone health of the load group, to some extent. Stress-relaxation, culture medium, and histology analysis are being done for a deeper understanding of the unexpected bone behavior observed. More studies are required to address the small sample size limitations, as well as other possible sources of error, to confirm if this 3D-printed bioreactor system can be used (or not) for long-term *ex vivo* organ cultures.

References

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