Compressive Stiffness Measurement of Trabecular Bone Surrogates in a Bioreactor

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INTRODUCTION: The stiffness of a material is quantified as the material's response to resist deformation due to an applied load and can be used to characterise a material's behaviour, and the measurement method, when tested under different conditions. Previous studies have been performed to test the stiffness of trabecular bone cores in bioreactors such as the ZETOS bioreactor chamber [1]. With the redesign of the ZETOS bioreactor chamber used by Vivanco et al. [1], a calibration method is required to ensure that accurate mechanical properties are measured in and out of the bioreactor. Therefore, the objective of this study was to investigate different rigid polymers' stiffness in and out of the bioreactor and determine if the bioreactor design has a significant effect on measured stiffness.

METHODS: For this study, two trabecular bone surrogates were investigated to compare stiffness in and out of the ZETOS bioreactor chamber, Ultem® (polyetherimide) and acetal copolymer (polyoxymethylene). Material samples with dimensions 10 mm×10 mm (height×diameter) were tested using a Bose ElectroForce 5500 (TA Instruments) at room temperature. These materials and dimensions were chosen to mimic trabecular bone cores without buckling [2], while also ensuring the samples fit within the bioreactor. Bioreactors were incubated at 37°C for 48 hours before testing to imitate *in vivo* temperature conditions. Parallel-surface and ball-and-socket adapters were used to apply compressive loads to samples while in and out of the ZETOS bioreactor. Prior to testing samples, LB 8801 silicone lubricant (Loctite) was applied to minimize friction between the x-rings and pistons. Three samples of each material were pre-conditioned using cyclic loading between 5 – 10 N force applied at 0.5 Hz for 5 cycles. After pre-conditioning, force and displacement measurements were recorded while samples were compressed to a maximum load of 150 N at a rate of 2 N/s and then unloaded. Tests were performed on each sample five times in and out of the bioreactor. The total stiffness of the system was calculated as the force over deflection. For each sample, a two-tailed t-test was performed assuming unequal variance and a significance level of 5% to compare the stiffness in and out of the bioreactor chamber using Microsoft Excel 2016.

RESULTS: Force-deflection data were observed to be similar for each material when tested in and out of the bioreactor (Figure 1a). The stiffness values, shown in Figure 1b, for the acetal copolymer showed no significant difference between being tested in and out of the bioreactor chamber ($p > 0.05$). The stiffness of the Ultem® polymer measured in the bioreactor was not significantly different than out of the bioreactor ($p > 0.05$) (Figure 1b).

DISCUSSION: These experiments demonstrated for two polymers, that their stiffness was not affected by the bioreactor. The bioreactor increased the testing system compliance resulting in an underestimation of sample stiffness; however, the friction between the piston and the bioreactor seals caused an overestimation of the sample stiffness. The bioreactor could cause an increase in trabecular bone surrogate stiffness if tests are performed without lubricant and at lab temperature. Limitations of the current study included the fast cooling rate of bioreactors during testing and the linear model used to calculate the stiffness of samples. For future tests, temperature control could be adopted, a model should be used to estimate sample stiffness, and nonlinear viscoelastic models can be used to test accuracy of mechanical behaviour even when samples are tested in a bioreactor.

SIGNIFICANCE/CLINICAL RELEVANCE: The results observed are significant as they provide initial steps toward calibrating mechanical loading systems and bioreactors such as the ZETOS bioreactor. Calibration ensures that the material stiffness, when tested in the bioreactor, is a true measure of the material's properties.

REFERENCES:

1. Vivanco, J., et al., *Apparent elastic modulus of ex vivo trabecular bovine bone increases with dynamic loading.* Proceedings of the Institution of Mechanical Engineers Part H-Journal of Engineering in Medicine, 2013. **227**(8): p. 904-912.
2. Zhao, S., et al., *Standardizing compression testing for measuring the stiffness of human bone.* Bone Joint Research, 2018. **7**(8): p. 524-38.

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IMAGES AND TABLES:

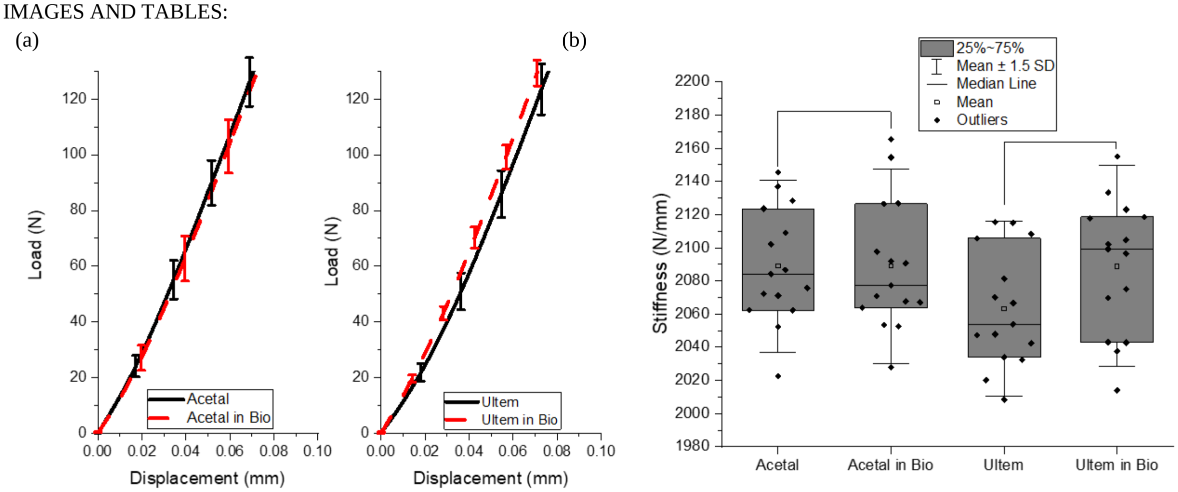

Figure 1 (a) Average force-deflection curves (± standard deviation) of the acetal and Ultem® polymers in and out of the bioreactor. (b) Total stiffness comparison between testing in and out of the bioreactor for the acetal and Ultem® polymers.