Engineered Bone Analog Models Designed for Testing on the International Space Station

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INTRODUCTION:
Aged individuals and astronauts experience bone loss despite rigorous physical activity. Bone mechanoresponse is in part regulated by mesenchymal stem cells (MSCs) that respond to mechanical challenge. Daily application of low intensity vibration (LIV) recovers MSC proliferation in senescence and simulated microgravity models, suggesting reduced mechanical signal delivery to MSCs may contribute to declining bone mechanoresponse in aging. To investigate Low Intensity Vibration (LIV) as an effective countermeasure to bone loss in microgravity, we developed 3D Bone Marrow Analogs (BMAs) to be maintained in hardware aboard the International Space Station (ISS). We engineered a perfusable 'Cube Well' system containing 16 BMAs housed within a CubeLab™ containing fluidics for automated cell culture and bioreactor system that can apply LIV. We expect LIV to increase anabolic activity of Bone Marrow Stromal Cells (BMSCs) in BMAs to be tested in ground controls and on the ISS.

METHODS:
To create a BMA model, we generated two idealized 3D trabecular lattices mimicking young (25%) or old (13%) trabecular bone volumes and filled them with hydrogel-encapsulated primary bone marrow MSCs to mimic bone marrow. We quantified the cellular mechanical environment under LIV treatment utilizing experimentally-calibrated finite element models for both individual BMA constructs and for the entire bioreactor system. Following cell viability and LIV-response studies, two CubeLab™ units, one with LIV bioreactor system and one without were designed to support automated media changes and preservation steps of BMAs on the ISS [4 Cube Wells/CubeLab™] along with ground controls. Verification tests required for autonomous experiments on the ISS included, quantifying the controlled release of osteogenic factors to BMAs with StemBeads®, testing diffusion rates in and out of the BMAs to assess media change frequency, determining solution change schedules for optimal fixation and RNA yields, assessing extent of mineralization, and verifying full-system integration, fluidics and accuracy of LIV delivery.

RESULTS:
The Cube Wells satisfied some/most mission success criteria in pre-launch testing. A subpopulation of MSCs in BMAs attached to the lattice and produced matrix; others remained in bulk hydrogel with high viability. Media changes every 2 days maintain cellularity. Small solutes in BMA exchanged by diffusion by 60 mins. BMAs with 13% bone volume generated 46% lateral strain along the LIV direction when compared to BMAs with 25% bone volume (**Fig. 1**, $p<0.0001$). Initial experimentation utilizing growth media shows that LIV increased Collagen-I in both 13% and 25% BMAs by 87% and 53% respectively ($p<0.01$) and +LIV 25% remained significantly higher compared to +LIV 13% group (37%, $p<0.05$). When comparing LIV and non-LIV groups, LIV increased cellularity by 50% and F-actin volume by 3-fold in 25% BMAs but not in 13% BMAs. Mineralization is being assessed after addition of osteogenic factors via StemBeads®.

DISCUSSION:
In this study we have developed a bi-phasic bone analogs with fully characterized hydrogel strain environment during low intensity vibrations. Our findings show that trabecular densities associated with advanced age (i.e. 13%) results in higher hydrogel strains while trabecular densities expected at adulthood (i.e. 25%) results in smaller strains during LIV. Despite the lower strain magnitudes, 25% BMAs are able to support higher cell proliferation, F-actin generation and collagen production under LIV, which may be due to proximity of MSCs to scaffold surfaces.

SIGNIFICANCE/CLINICAL RELEVANCE:
BMA model and the generation of the Cube Well & CubeLab™ systems will not only allow us to test the efficacy of LIV on combatting bone loss during microgravity/space flight but also provide a valuable platform for studying bone marrow mechanobiology and bone related diseases on board of ISS.

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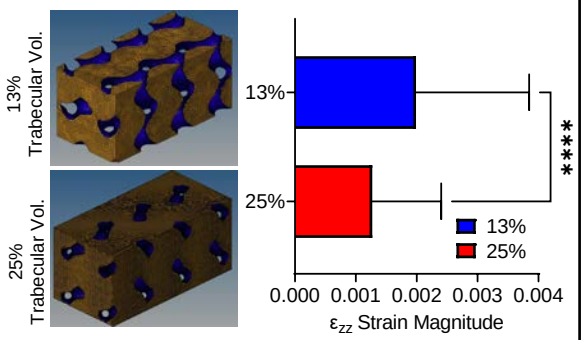

Fig.1 3D strain distribution between 13% and 25% scaffolds under LIV. Comparing the lateral ε_{zz} magnitude showed that ε_{zz} of 13% BMA was 46% larger compared to 25% BMA ($p<0.0001$).

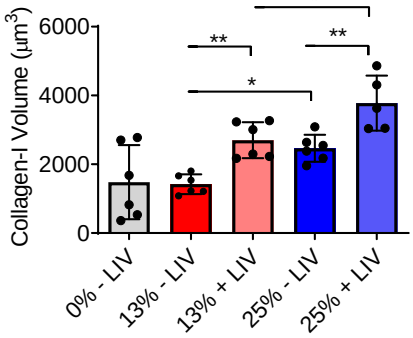

Fig.2 Comparing Collagen-I volume, 0% and 13% were not different while -LIV 25% group had a 73% increase compared to -LIV 13% group ($p<0.05$). LIV increased Collagen-I in both 13% and 25% groups by 87% and 53% respectively ($p<0.01$).