Hydrogel Microencapsulation Mimics Whole Body Response to Low Magnitude High Frequency Vibration

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Statement of Purpose: Low-magnitude, high-frequency (LMHF) vibrations show anabolic effects on bone when applied to the whole body in both animal and human studies.[1] In humans, whole-body vibrations have shown modest increases in the bone mineral density of postmenopausal women, children and adolescents, while showing little effect in young adults, prompting large scale long term studies to establish optimal vibration parameters.[2] There are conflicting results when LMHF vibrations are applied to cell culture, with some studies showing no effect and others showing increased osteodifferentiation of progenitor cells.[3] Studies using 2D monolayer on tissue culture plastic tend to report no effect, while studies using 3D culture in scaffolds tend to report progenitor osteodifferentiation.[4] The majority of scaffolds used in such studies are from natural sources, which in and of themselves may promote differentiation due to biochemical and microarchitectural cues. This study evaluates the effect of LMHF vibrations on human mesenchymal stem cells (hMSCs) microencapsulated within the synthetic polymer polyethylene glycol diacrylate (PEGDA), which has no inherent cues and serves as a blank slate to encapsulated cells. Herein, we assay entrapped cells for adipocyte, chondrocyte and osteoblast differentiation following LMHF vibration.

Methods: Hydrogel precursor solution containing hMSCs, 10 kDa PEGDA (10% w/v) in HEPES buffered saline and hydrophilic photoinitiators (0.1 mM eosin Y and 1.5% w/v triethanolamine) was prepared as previously described.[5] A hydrophobic photoinitiator solution (2,2-dimethoxy-2-phenyl acetophenone in 1-vinyl-2-pyrrolidinone; 300 mg/ml) was prepared then added to mineral oil (3 µl/ml). Microspheres were formed by adding hydrogel precursors to the mineral oil solution and photopolymerizing a vortex-induced emulsion. Microspheres were isolated, placed in 25 cm^2 flasks and exposed to 0.3, 3, and 6 g vibrations with a magnitude of 0.00745, 0.0745, and 0.149 mm, respectively, at 100 Hz for 24 h at room temperature using a BOSE ElectroForce 3100 mechanical testing machine. Control microspheres were not vibrated and kept at room temperature for 24 h. Microencapsulated hMSCs were assessed for cell viability on days 1, 3, 7, 14, and 21 using calcein-AM and ethidium homodimer-1 stains to label live and dead cells, respectively, which were then observed under a Zeiss epifluorescent microscope. Lineage specific assays evaluated hMSC differentiation using Oil red O, Alizarin red, alkaline phosphatase, and Safranin O staining to detect adipocytes, calcium mineralization, osteoblasts, and chondrocytes, respectively.

Results: Cell viability started at approximately 80% for all groups. By the end of the study, viability had dropped to about 48% (Fig. 1). Viability appeared to decrease over time as calcification increased. All (both control and vibrated) microencapsulated hMSCs stained negative for Alizarin Red S, Saffranin O, and Oil red O stains on Days 1-7. Only Alizarin Red S stained positive on Days 14-21 (Fig. 2), while alkaline phosphatase AP activity was observed on Day 4 (Fig. 3a). In 0.3 g and 3 g vibrated samples, mineralization occurred a week faster than unvibrated controls (Fig. 3). By Day 21, mineralization in controls caught up to that in vibrated samples.

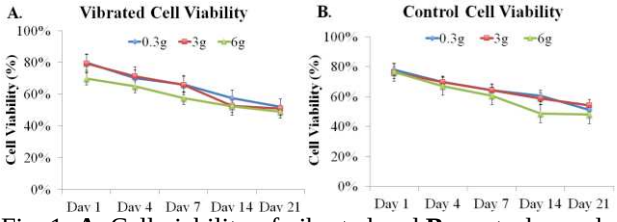

Fig. 1: **A.** Cell viability of vibrated and **B.** control samples from Day 1 to Day 21. All samples decreased in viability over time. There was no statistical difference between samples on any days. Error bars show standard deviation.

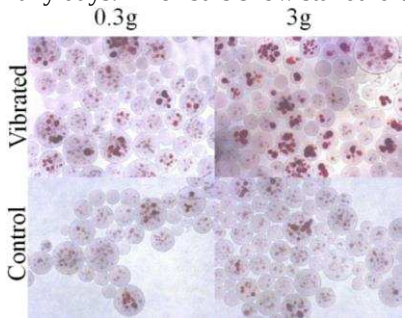

Fig. 2: Alizarin Red S positive stain for 0.3 g and 3 g test and control microspheres on Day 14 (magnification 10x).

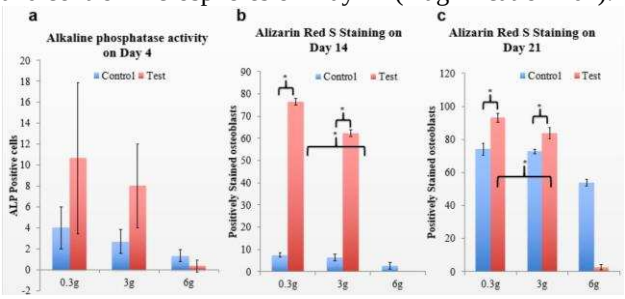

Fig. 3: **a.** Alkaline phosphatase activity and **b,c.** Alizarin Red S results. Asterisks show significance ($p < 0.05$). Error bars show standard deviation. * show statistical significance; $p<0.05$. Error bars show standard deviation.

Conclusions: Low g vibration appears to accelerate osteogenic differentiation of PEGDA microencapsulated hMSCs while high g vibration appears to inhibit it. Thus, a synthetic 3D cellular microenvironment without any additional cues recovers the osteoinductive effects of LMHF vibrations in the in vitro environment.

References:

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