# Improved Human Bone Marrow Mesenchymal Stem Cell Osteogenesis in 3D Bioprinted Tissue Scaffolds with Low Intensity Pulsed Ultrasound Stimulation

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## Introduction

3D printing and ultrasound techniques are showing great promise in the evolution of human musculoskeletal tissue repair and regeneration medicine. The uniqueness of the present study was to combine low intensity pulsed ultrasound (LIPUS) and advanced 3D printing techniques to synergistically improve growth and osteogenic differentiation of human mesenchymal stem cells (MSC). Specifically, polyethylene glycol diacrylate bioinks containing cell adhesive Arginine-Glycine-Aspartic acid-Serene (RGDS) peptide and/or nanocrystalline hydroxyapatite (nHA) were used to fabricate 3D scaffolds with different geometric patterns via novel table-top stereolithography 3D printer. These results illustrate the effectiveness of the combination of LIPUS and biomimetic 3D printing scaffolds as a valuable combinatorial tool for improved MSC function, thus make them promising for future clinical and various regenerative medicine application.

## Overview and characterization

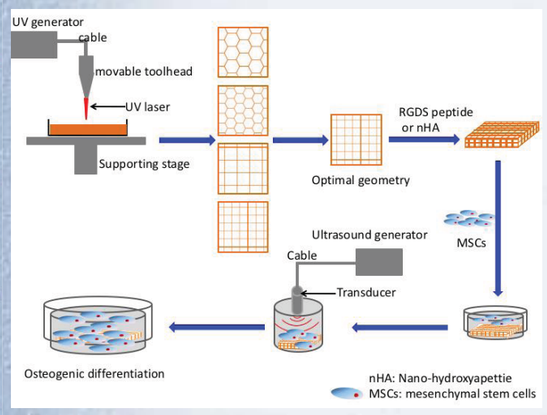



Figure 1. Schematic diagram of the research

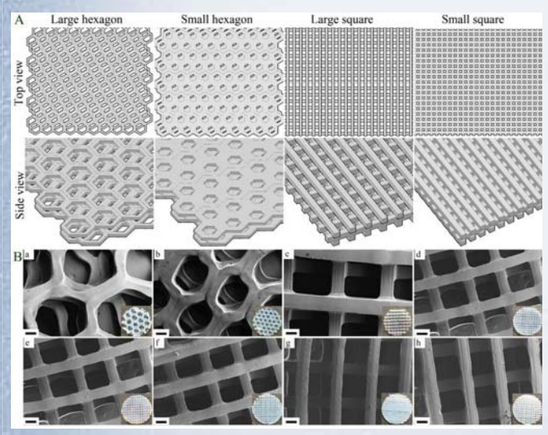



Figure 2. (A) CAD models of four bone scaffolds with large hexagonal (LH), small hexagonal (SH), large square (LS) and small square (SS) pore structure. (B) SEM images of various 3D printed scaffolds: (a) LH; (b) SH; (c) LS; and (d) SS pore structure. SEM images of small square pore scaffolds with or without RGDS and nHA: (e) PEGDA; (f) PEGDA-RGDS; (g) PEGDA- nHA; and (h) PEGDA-nHA-RGDS scaffolds. The insert images are the corresponding scaffolds' photographs. Scale bar = 100 μm.

## Cell proliferation

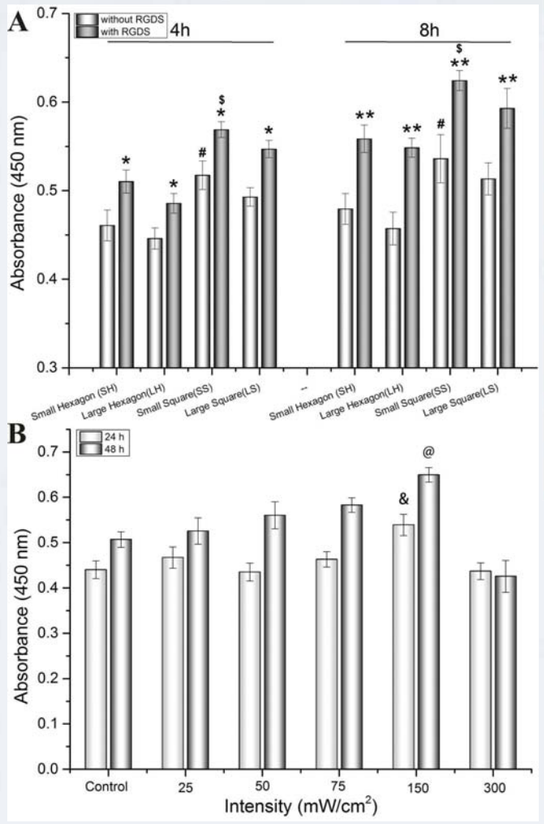



Figure 3. (A) 4 h and 8 h MSC adhesion on 3D printed scaffolds with and without RGDS, (B) 48 h MSC growth on the 2D culture plates under varied LIPUS treatment conditions. Data are mean ± standard error of the mean, n = 9.

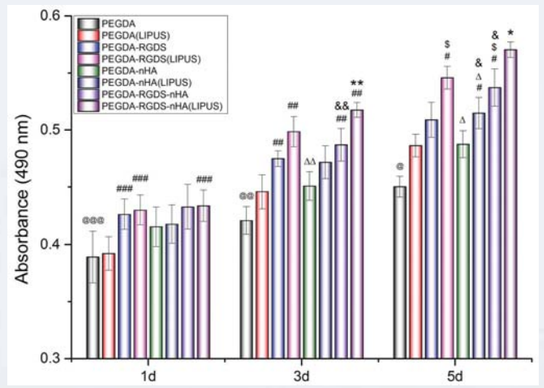



Figure 4. Enhanced MSC proliferation on 3D printed scaffolds with excitation after 1, 3 and 5 days of cultures. Data are mean ± standard error of the mean, n=9.

Figure 5. Confocal microscopy images of MSC proliferation on 3D printed scaffolds with and without LIPUS treatment after 5 days culture. (A) PEGDA without and (B) with LIPUS; (C) PEGDA-RGDS without and (D) with LIPUS; (E) PEGDA-nHA without and (F) with LIPUS; and (G) PEGDA-RGDS–nHA without and (H) with LIPUS, respectively. The cytoskeleton and cell nuclei were stained by Texas Red®-X phalloidin (red) and DAPI (blue), respectively.

## Osteogenetic differentiation

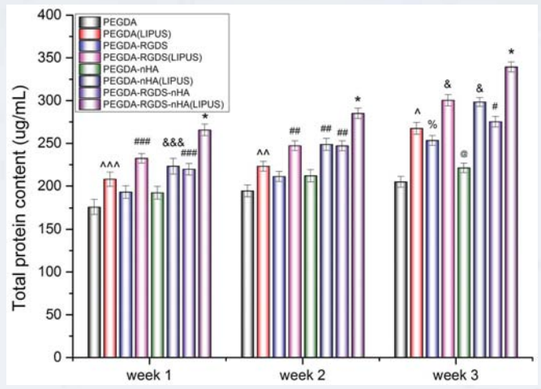



Figure 6. Improved total protein content on 3D printed bioactive scaffolds with LIPUS treatment after three-week MSC osteogenic differentiation. Data are mean ± standard error of the mean, n = 6.

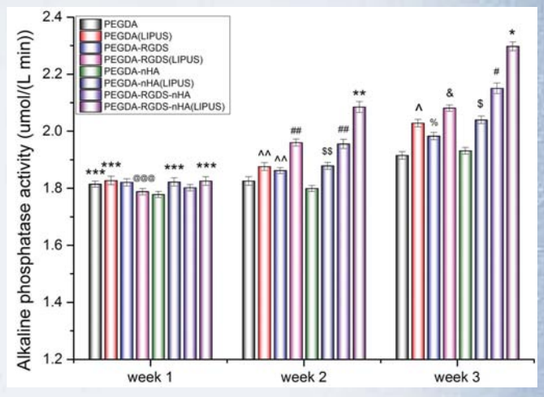
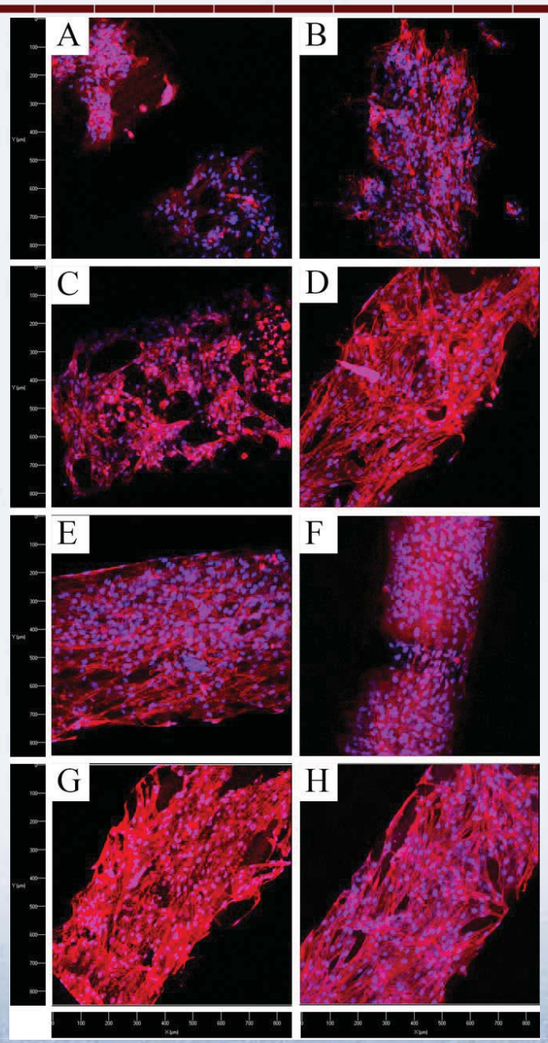



Figure 7. Greatly enhanced alkaline phosphatase activity on 3D printed bioactive scaffolds with LIPUS treatment after three-week MSC osteogenic differentiation. Data are mean ± standard error of the mean, n = 6.



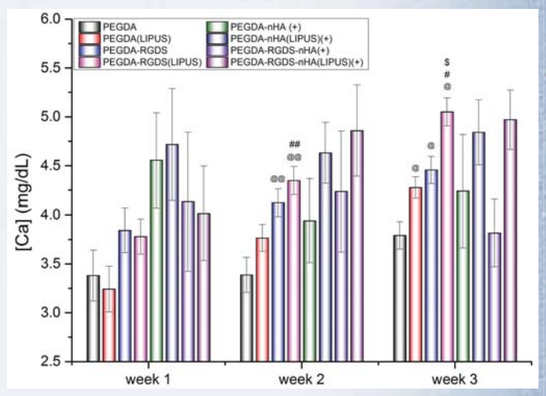




Figure 8. Calcium deposition on 3D printed bioactive scaffolds with and without LIPUS treatment after three-week MSC osteogenic differentiation. Data are mean ± standard error of the mean, n = 6.

## Conclusion

In the current work, RGDS and/or nHA-containing PEGDA 3D scaffolds with various geometric patterns were successfully printed by our novel table-top stereolithography 3D printer. Small square pores proved to be the optimal geometric pattern for MSC adhesion with optimal LIPUS excitation parameters of 1.5 MHz, 20 % duty cycle with 150 mW/cm2 intensity. MSC proliferation and osteogenic differentiation demonstrate that RGDS and nHA readily enhance the bioactivity of PEGDA scaffolds. Additionally, our results also show that LIPUS can further promote MSC proliferation while enhancing osteogenic differentiation. Therefore, integrating LIPUS and 3D printing bioactive scaffolds can provide a valuable combinatorial tool for improved MSC growth and osteogenic differentiation, thus making them promising for future regenerative medicine applications.

## Acknowledgments

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