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# Bone tissue regeneration using 3D printed microstructure incorporated with hybrid nano hydrogel

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### **Background & Objective**

Three-dimensional (3D) functional constructs with biomimetic and biomechanical properties are ideal for various tissue regeneration. Aforementioned properties of 3D fabricated constructs mainly depend on the intrinsic attribution of used materials and the fabrication methods. In this respect, current use of hydrogels for damaged tissue repair in the musculoskeletal tissue are not perfect due to the lack of suitable mechanical properties, as well as the high biomimetic requirement for successful regeneration. To overcome the drawback, we developed a novel functionalized hydrogel with bioactive gold nanoparticles (GNPs) and further reinforced with highly mechanical strength microstructure via a fused deposition modeling (FDM) 3D printer for bone tissue regeneration.



**Figure 1. Variations in tissue stiffness**, from Ref. [Disease Models and Mechanisms 4.2 (2011): 165-178]

## Scaffold design and Fabrication





Characterization



Wavelength, nm



**Figure 5. Viability of ADSCs** in the presence of RGD, GNP and GNP-RGD at different concentrations.



**Figure 6. Compressive modulus** of 3D printing PLA scaffolds, Mandible, and Gelatin hydrogel. (Data of mandibular bone reproduced from ref.) There are no significant differences between PLA scaffold and PLA infilled with hydrogel and GNP.

### **Osteogenic Differentiation**





**Figure 8. Deposition degree of calcium** from ADSCs culture in the Gel, Gel-GNP, and Gel-RGNP at 21 days. (a) Optical images of ARS stained complex structures and (b) quantification of mineralization by UV spectrometer. "\*\*" indicates significant difference of p < 0.01.



**Figure 9. Gene expressions levels** of ADSCs culture in the Gel, Gel-GNP, and Gel-RGNP at 7 and 14 days for osteogenic differentiation markers: (A) COL1, (B) ALP, (C) RUNX2, and (D) BSP. Relative gene expression of each gene (mean  $\pm$  SD), normalized to the expression of the housekeeping gene GAPDH, are compared with ADSCs cultured in standard cell culture conditions. "\*" indicates significant difference of *p* < 0.05. "\*\*" indicates significant difference of *p* < 0.01.

**Figure 2.** Patient-specific Bone tissue regeneration using 3D Printing PLA incorporated with hybrid hydrogel composed of gold nanoparticles and human adipose derived stem cells



Figure 3. (A) Pre-designed 3D scaffolds and (B) their porosity

\* Data of mandibular bone reproduced from Ref. [Journal of biomechanics 34 (2001): 799-803]



Figure 7. The effect of altering hydrogel component with the 3D printing PLA structure on ADSC spreading and proliferation. (A) optical images of ADSC spreading outside and inside of the hydrogel structures. (B) Actin cytoskeletal organization of representative ADSCs to exist their outside after 24h of seeding. Scale bars =  $100 \ \mu m$ . (C) Cell outlines of twelve representative ADSCs to exist their inside after 24h of seeding. Scale bars =  $100 \ \mu m$ . (C) Cell outlines. Scale bars =  $100 \ \mu m$ . (D) Quantification of ADSCs spreading area of the hydrogel inside. (E) Proliferation of ADSCs cultured on the Gel, Gel-GNP, and Gel-RGNP, investigated by CCK.

#### Conclusion

In conclusion, we have successfully designed and developed a novel functional hydrogel incorporating cRGD conjugated GNPs (RGNPs), which reinforced the mechanical strength through the combination of 3D printed microstructure and hydrogel. The stiffness of the composite microstructure was significantly increased to a similar level of native bone tissue. Before cell encapsulation, the cell viability tests suggest that all components not only had no significant cytotoxic effects but also, RGNPs showed and increased cell proliferation with dose-dependent manner. Moreover, the addition of RGD to the composite constructs enhanced the cellular behavior. The in vitro experiments showed that encapsulated ADSCs in the Gel-RGNPs promoted significantly higher cell adhesion, spreading and proliferation, which led to an increased gene expression of osteogenic specific markers. Our findings suggest that reinforced composite hydrogels can be a useful strategy for improving the bone tissue regeneration.