PosterGate

3D Printed Scaffold With Hierarchical Biomimetic Structure For Osteochondral Regeneration

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Introduction

Articular osteochondral repair and regeneration are a challenging problem worldwide due to the extremely weak inherent regenerative capacity of the tissue. Currently, the gold standard surgical procedures for treating chondral lesions are autologous cartilage transplantation or autologous chondrocyte implantation, etc. However, this approach is still not perfect due to limited resources of cartilage tissue. In addition, TGFβ1 and nano hydroxyapatite (nHA) play a crucial role in chondrogenesis and osteogenesis, respectively. Here, we firstly prepared TGFβ1 loaded PLGA nanoparticles (TPNPs) by coaxial electrospray method. Next, we fabricated 3D bioprinted gelatin methacrylate–polyethylene glycol diacrylate (GelMA-PEGDA) scaffolds with biphasic TPNPs and nHA distributions, and then investigated the effects of this scaffold on the growth and osteochondral differentiation of human bone marrow mesenchymal stem cells (hMSCs).

Overview and characterization
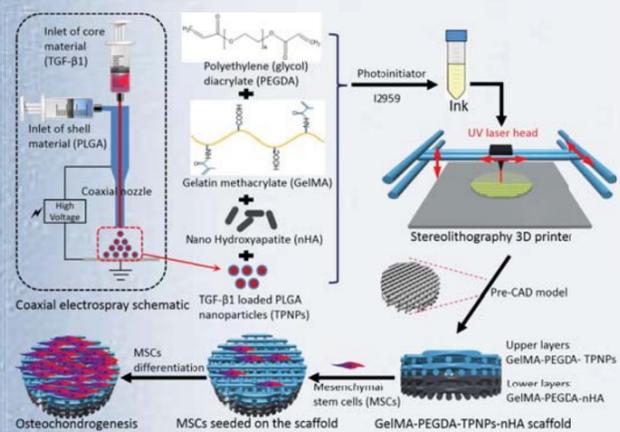
Figure 1. Schematic diagram of the research
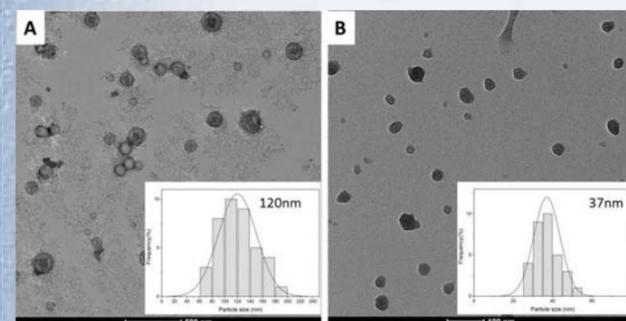
Figure 2. Transmission electron microscope (TEM) images of (A) TGF-β1 loaded PLGA NPs and (B) blank PLGA NPs. The inset images are the size distribution of the corresponding nanoparticles.

Figure 3. Scanning electron microscope (SEM) images in top and bottom views of (A) GelMA-PEGDA-nHA/TGF-β1 PLGA NPs, (B) GelMA-PEGDA-nHA/bare TGF-β1, and (C) GelMA-PEGDA-nHA/blank PLGA NPs scaffolds. Scale bar = 200 μm. The inset images are photographs of the corresponding scaffolds. (D)SEM image of cross-sectional view between GelMA-PEGDA-nHA and GelMA-PEGDA- blank PLGA NPs, Scale bar = 100 μm. (E) Side and (F) top views of the CAD 3D scaffold model. (G) Microscope image and (H) surface plot of 3D printed scaffold. The element analysis (I-J) and the diagram results (K) of lower and upper layers of scaffolds. **p<0.01 represented significantly differences between them.

Cell proliferation
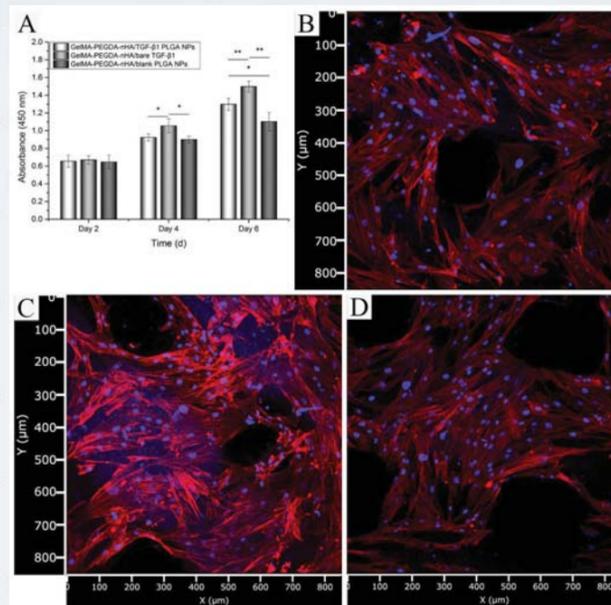
Figure 4. (A)Proliferation (2, 4 and 6 days) and confocal micrographs (Day 6) of hMSCs grown on (B) GelMA-PEGDA-nHA/TGF-β1 PLGA NPs, (C) GelMA-PEGDA-nHA/bare TGF-β1, and (D) GelMA-PEGDA-nHA/blank PLGA NPs scaffolds. The cytoskeleton and cell nuclei were stained with Texas Red®-X phalloidin (red) and DAPI (blue), respectively. Data are mean ± standard deviation, n = 8. *p < 0.05 and **p < 0.01 when compared to all other groups at each time points.
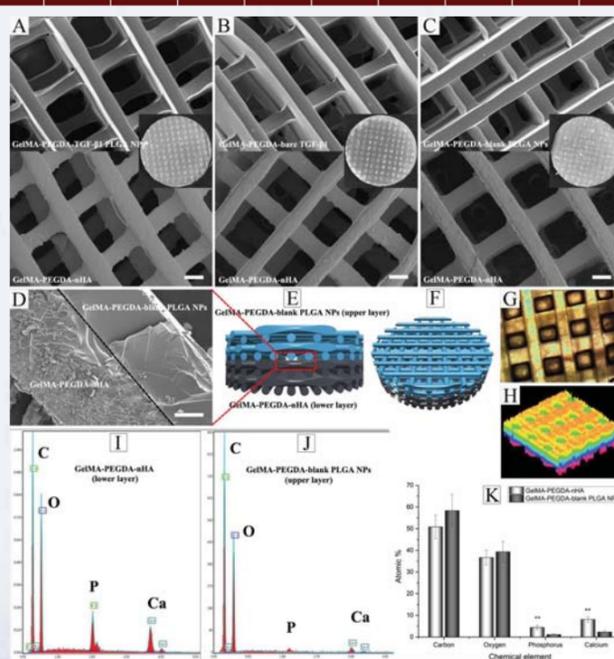
Osteogenetic and chondrogenic differentiation
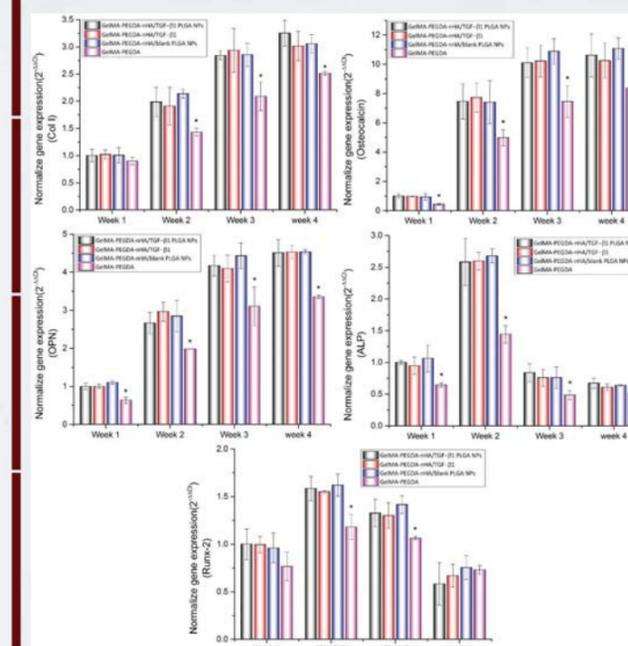
Figure 5. Normalized gene (Col I, Osteocacin, OPN, ALP, and Runx-2) expressions of hMSCs after osteogenetic differentiation on various scaffolds over 4 weeks. Data are mean ± standard deviation, n = 8. *p < 0.05 and **p < 0.01 when compared to all other groups at each time points.

Figure 6. Alizarin stained micrographs of hMSCs after osteogenic differentiation on the surface of the scaffolds with different components for week 2 and 4. Scale bar = 200 μm.
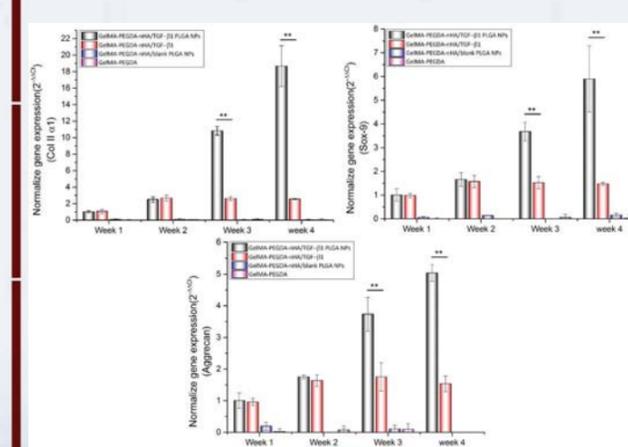
Figure 7. Normalized gene expressions (Col II α1, Sox-9, and Aggrecan) of hMSCs after chondrogenic differentiation on various scaffolds over 4 weeks. Data are mean ± standard deviation, n = 8. *p < 0.05 and **p < 0.01 when compared to all other groups at each time points.
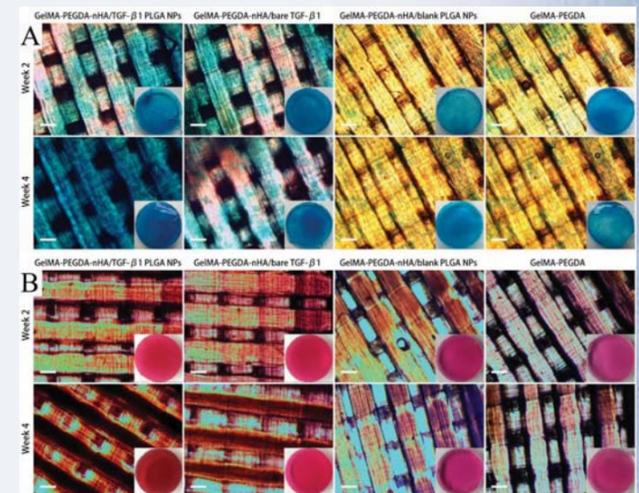
Figure 8. (A)Alcian Blue and (B)Safranin O stained micrographs of hMSCs after chondrogenic differentiation on the surface of the scaffolds with different components for week 2 and 4. Scale bar = 200 μm.

Conclusion

Comprehensively, TGF-β1 loaded PLGA NPs with 120 nm particle size were procedurally prepared by co-axial electrospraying technology. GelMA and nHA was successfully synthesized by chemical modification and hydrothermally method, respectively. GelMA-PEGDA was employed as elementary ink in this study. Finally, nHA and TGF-β1 PLGA NPs were distributed separately in in lower and upper layers to fabricate biomimic biphasic scaffolds (GelMA-PEGDA-nHA/TGF-β1 PLGA NPs) using our 3D stereolithography-based printer. A customizable 3D printed GelMA-PEGDA scaffolds with biphasic TPNPs and nHA distributions structure were prepared successfully. The scaffold provided a excellent platform for MSC proliferation and osteochondral differentiation. The most significant improvement in chondrogenic gene (Col II α1, Sox-9, Aggrecan) and osteogenesis gene (Col I, Osteocalcin, OPN, Runx-2, ALP) expressions were observed on the 3D scaffolds. This study demonstrated that customizable 3D printed scaffolds are excellent candidates for promoting osteochondrogenesis differentiation of hMSCs, thus promising for future cartilage regenerative medicine applications.

Acknowledgments