Research Week 2022

Biomimetic nanoscale mineralization of bioprinted cell-laden microgels

Mauricio Sousa[1], Gabriela Balbinot[2], Rauhl Visalakshan[1], Ramesh Subiah[1], Luiz Bertassoni.[1,3,4,5]

1 Division of Biomaterials and Biomechanics, Department of Restorative Dentistry, OHSU School of Dentistry
2 Universidade Federal do Rio Grande do Sul – UFRGS, School of Dentistry, Dental Materials Department, Porto Alegre, RS, Brazil.
3 Center for Regenerative Medicine, OHSU School of Medicine.
4 Department of Biomedical Engineering, OHSU School of Medicine.
5 Cancer Early Detection Advanced Research (CEDAR), Knight Cancer Institute.

Keywords

Bioprinting, microgels, mineralization, bone regeneration, osteocytes.

Abstract

Introduction: The bone tissue is a complex nanocomposite, organized in a calcified extracellular matrix. Bone autografts remain the gold standard to treat traumatic bone injuries. However, autografts can increase morbidity of the patient's donor site and has limited availability. On the other hand, bioprinted microgels are attractive biomaterials that offer injectability, tunability, and acts as a cargo to deliver therapeutic proteins and cells. Here, we aim to bioprint and mineralize cell-laden gelatin methacrylate (GelMA) microgels and test the effects of cryopreservation on cell viability for bone tissue engineering applications.

Methods: A 10% W/V gelMA was 3D printed (600 μm^2 square shaped) microgels in a digital light processing 3D printer. For three days, these structures were mineralized with 9 mM $CaCl_2.2H_2O$, osteopontin (100 ug/mL), 4.2 mM K_2HPO_4, and Alpha-MEM media. The microgels were characterized by scanning electron microscopy (SEM), transmission electron microscopy (TEM), Fourier transform infrared spectroscopy (FTIR), alizarin red (AR), and von Kossa staining (VKS) for effective nanoscale mineralization. The ability of these microgels to maintain functional osteocytes (1 $x10^6$ cell/mL) post mineralization and cryopreservation (3 days) was evaluated by live and dead stain and sclerostin immunostaining.

Results: GelMA microgels could be mineralized and the rich mineralized areas were confirmed by SEM, TEM, EDAX, AR, and VKS. Mineralized microgels presented higher rates of nanocrystalline $CaPO_4$, as confirmed by apatite phosphate peaks in FTIR spectra. Live and dead stains showed osteocyte viability after mineralization for 3 days and cryopreservation

for 1 and 3 days. Also, sclerostin was highly expressed in cryopreserved mineralized microgels, indicating that the mineralization effectively maintains osteocytes function, and cryopreservation preserves the viability of osteocytes.

Conclusions: Mineralization of bioprinted gelMA microgels are efficient strategies to mimic the mineralization in nanoscale. These biomaterials can be cryopreserved with osteocytes, which may validate their translation for bone tissue engineering approaches.