Three-dimensional (3D) bone tissues derived from stem cells as a novel model for mineralization

Poster Number 23

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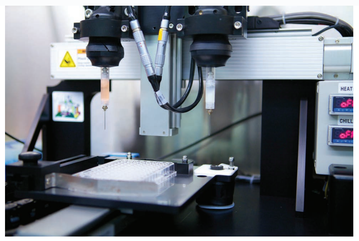
Abstract

When compared to standard two-dimensional (2D) monolayers, three-dimensional (3D) systems have been shown to more accurately mimic the physiologic cellular microenvironment across a variety of tissues. We have previously reported successful fabrication and mineralization of bioprinted 3D structures comprised of primary bone marrow-derived stem cells (BMMSCs) and endothelial cells (ECs). Here we present a novel 3D tissue printing method using the Novogen MMX Bioprinter® platform and bioink formulated with a lower cell density to model mineralization. The approach was validated first in 3D bone containing BMMSCs and ECs, with successful mineralization demonstrated by alizarin red staining. Because the method requires fewer cells, it enables the use of more limited cell populations, such as those isolated or derived from diseased patients, to model disease phenotypes *in vitro*. Induced pluripotent stem cells (iPS cells), which can be generated from healthy and diseased patients, provide an ideal material for this application. In the current study, we incorporate iPS cell-derived MSCs and ECs from control patients into bioink and subsequently fabricate 3D tissues for mineralization studies. Tissues printed onto transwell surfaces displayed features of bone mineralization, as assessed by positive alizarin red or Von Kossa staining. Our results demonstrate the feasibility of incorporating iPS cell-derived MSCs and ECs from healthy individuals into bioink and subsequently fabricating 3D tissues for mineralization studies. These 3D bone tissues will be valuable for modeling genetic bone diseases *in vitro* such as fibrodysplasia ossificans progressiva (FOP), a rare disease of connective tissue resulting from a mutation in ACVRI (R206H) characterized by heterotopic ossification of soft tissue. This mineralization model will also be valuable for enabling future therapeutic, transplant, and drug development approaches that utilize engineered tissues.



The Novogen MMX Bioprinter®.

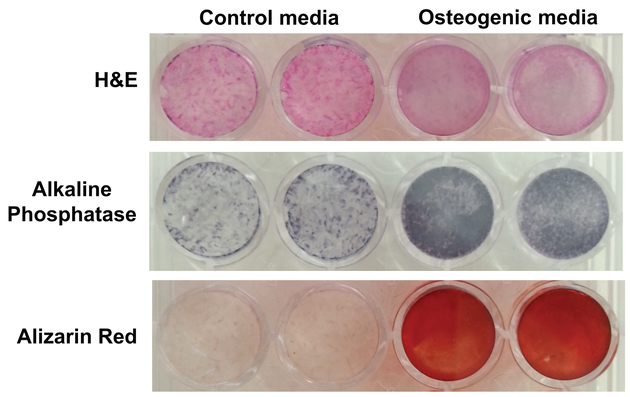


Figure 1: Demonstration of mineralization competency of BMMSC:EC co-cultures in 2D. Co-cultures of BMMSC and HUVEC mineralize effectively in the presence of osteogenic media, as evidenced by both increased alkaline phosphatase and Alizarin red stains

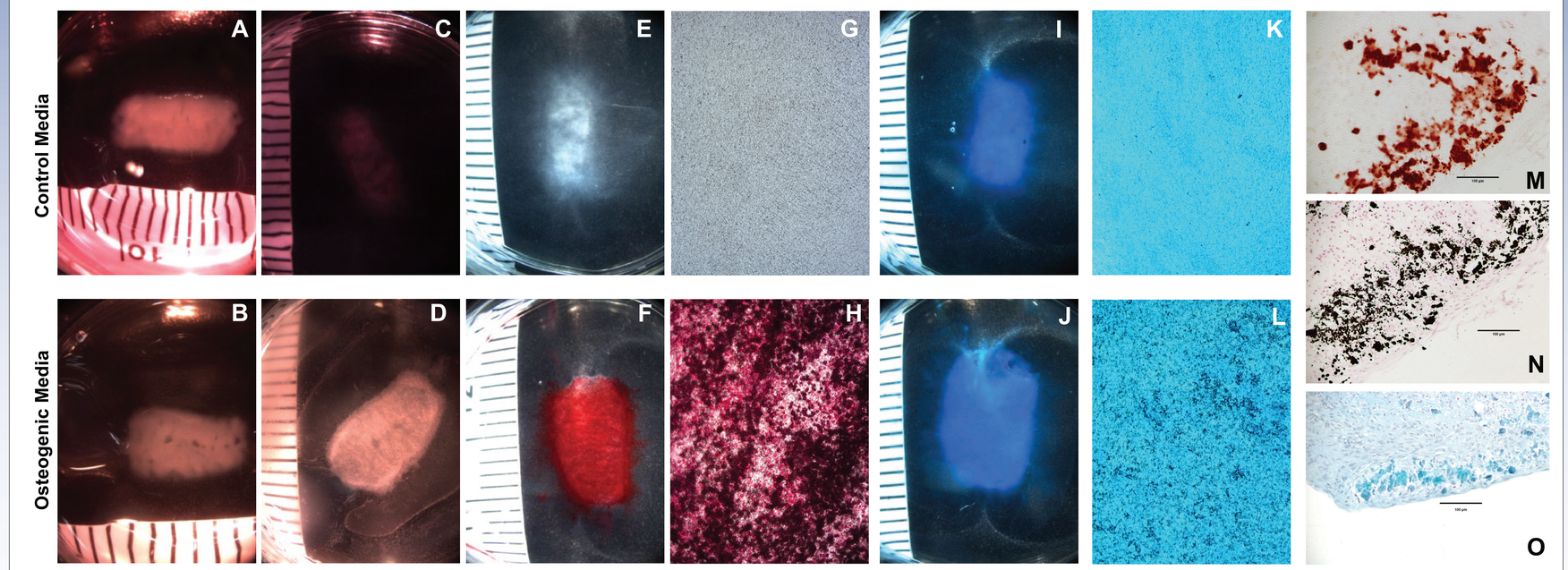


Figure 2. 3D bioprinted bone tissues derived from BMMSC and EC mineralize effectively. Tissues were similar in appearance at day 1 (A,B). Differences in macroscopic appearance and opacity were observed in tissues in osteogenic media (D) when compared to control media (C) at day 13. Tissues mineralized in osteogenic media stained positive for alizarin red at day 13 (F, H) while tissue in control media does not (E, G). Proteoglycan production was increased in osteogenic media (J,L) when compared to control media (I,K) by alcian blue staining. Mineralized tissues grown in osteogenic media stained positive for alizarin red (M), Von Kossa (N), and alcian blue (O) when analyzed histologically (20x images).

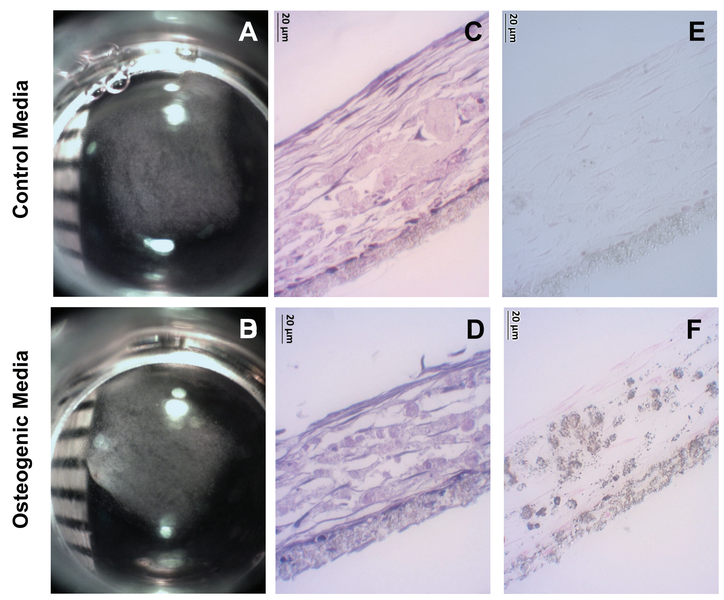


Figure 3. 3D bioprinted bone tissues can be derived from iPSC. Tissues comprised of iPSC-MSC and iPSC-EC were uniform by macroscopic appearance at day 1 in control and osteogenic media (A,B). H&E stain on bioprinted tissue at day 10 shows both rounded and fibrotic cell morphology the tissues, (C,D). Von Kossa staining at day 10 shows positive mineralization in tissues grown in osteogenic media (E, F), 60x images.

Materials and Methods

Cell Culture: Wild type BMMSC and HUVEC were obtained commercially from Lonza and Corning, respectively. iPSC derived-MSCs and ECs were prepared according to previously optimized protocols (modified from White, et al. Stem Cells. 2013 Jan;31(1):92-103).

Tissue Fabrication and culture: All tissues were fabricated directly onto the membranes of standard Corning transwell culture well inserts using standard Organovo bioprinting protocols and the Novogen Bioprinter® . Bioprinted tissues were incubated at 37ºC for up to 13 days in control media or OsteoMax-XF™ osteogenic media (Millipore).

Staining: After incubation, BMMSC:EC constructs were fixed in 10% NBF rinsed in PBS and whole mount stained with alizarin red. iPS cell-derived:EC constructs were fixed in 2% PFA solution and paraffin embedded. H&E and Von Kossa staining were performed using standard histological protocols.

Summary

- We have successfully generated mineralized tissue using a novel low cell density 3D printing method
- Bioink formulated out of BMMSC or IPS cell-derived MSCs can be used to fabricate 3D tissues for mineralization studies assessed by positive alizarin red and Von Kossa staining
- This printing approach provides a novel method to potentially model bone diseases in vitro