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RESEARCH ARTICLE

Fabrication of prevascularized co-culture spheroids and their 3D bioprinting application

Supplementary File

S1. Supplemental results and discussion

Given the known donor-to-donor variability in the differentiation potential of hMSCs,[1] it is theoretically possible that such variability also exists in their ability to support vasculogenesis. Therefore, in the present study, the ability of human umbilical vein endothelial cells (HUVECs) to form prevascular structures with human mesenchymal stem cells (hMSCs) from a different donor was evaluated. In aggregates formed from a co-culture of HUVECs and a second hMSC donor, CD31 staining showed that prevascular structures formed and persisted for 28 days when cultured in endothelial cell growth medium-2 (EGM-2; Figure S1a). When the aggregates were cultured in EGM-2 or EBM-2 with 100 ng/mL fibroblast growth factor 2 (FGF-2), both groups demonstrated extensive prevascular cord and plexus formation, as seen in CD31 staining at days 7 and 14 (Figure S1b).

Donor-to-donor variability is well described for hMSCs for orthopedic tissue engineering processes such as osteogenesis and chondrogenesis. However, less is known about donor-to-donor variability for hMSCs in vasculogenic processes. The current system was used with a second hMSC donor to demonstrate prevascular structure formation and persistence up to 28 days in EGM-2, as well as robust prevascular structure formation in aggregates cultured in either EGM-2 or EBM-2 supplemented with 100 ng/mL FGF-2. These reassuring results do not indicate significant donor-to-donor variability of hMSCs in the current system and suggest that therapies developed from it might be broadly applicable across the patient population.

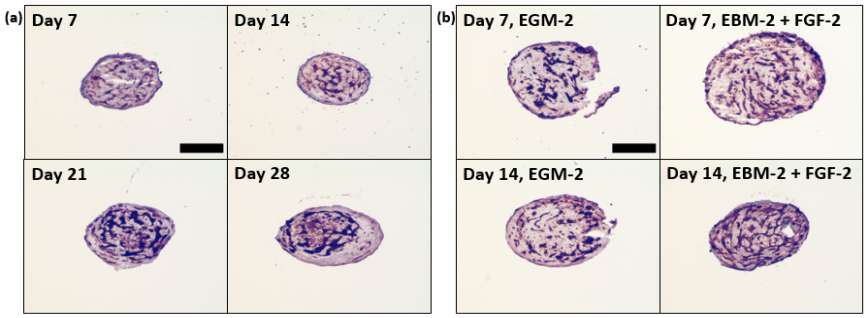


Figure S1. Prevascular structure formation with a second hMSC donor. (a) Prevascular structure formation was observed over four weeks of *in vitro* culture in EGM-2 with a different hMSC donor. (b) Robust prevascular structures were observed over two weeks when aggregates were cultured in either EGM-2 or EBM-2 supplemented with 100 ng/mL of FGF-2. Scale bars: 200 μm; magnification: 10×.

References

1. Phinney DG, Kopen G, Righter W, Webster S, Tremain N, Prockop DJ. Donor variation in the growth properties and osteogenic potential of human marrow stromal cells. *J Cell Biochem*. 1999;75(3)424-36.

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