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Running title: Prevascularization for 3D bioprinting

Supplementary File

Supplemental results

Given the known donor-to-donor variability of differentiation potential of hMSCs,[1] it is theoretically possible that such a variability exists in their ability to support vasculogenesis as well. Therefore, in the present study, the ability of HUVECs to form prevascular structures with 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 EGM-2 (Fig S1a). When the aggregates were cultured in EGM-2 or EBM-2 with 100 ng/mL FGF-2, both groups demonstrated extensive prevascular cord and plexus formation as seen in CD31 staining at 7 and 14 days (Fig S1b).

Supplemental discussion

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.

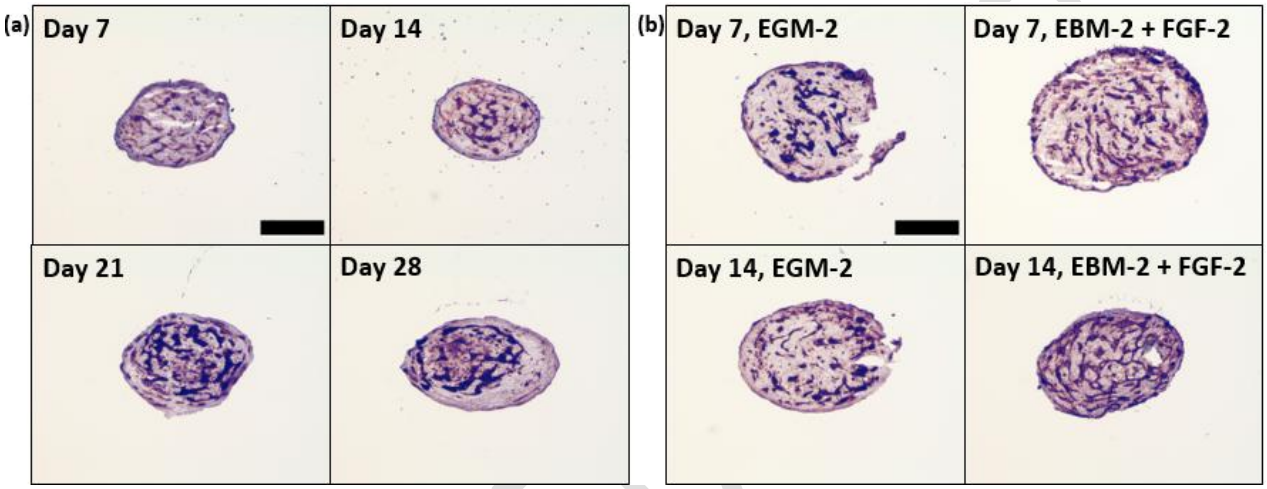

Figure S1. Prevascular structure formation with a second hMSC donor. (a) Prevascular structure formation was observed over four weeks of in vitro culture in EGM-2 with a different hMSC donor. (b) Robust prevascular structures were observed over two weeks when aggregates were cultured in either EGM-2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

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. doi: 10.1002/(SICI)1097-4644(19991201)75:3<424::AID-JCB8>3.0.CO;2-8