Osteogenic differentiation of ASC and MSC in modular protein/ceramic microenvironments

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Purpose: Modular tissue engineering is a rapidly developing field that applies a "bottom-up" approach to fabricate engineered tissues. Cell-seeded hydrogel microenvironments ("microbeads") can be individually cultured, differentiated, and then later combined to create macroscopic tissue constructs. In the current study, we seeded bone marrow mesenchymal stem cells (MSC) and adipose-derived stem cells (ASC) in collagen/fibrin (COL/FIB) and collagen/fibrin/hydroxyapatite (COL/FIB/HA) three-dimensional hydrogel microbeads and monitored their osteogenic differentiation.

Methods: Microbeads composed of 50/50 (mass ratio) COL/FIB were generated through a water-in-oil emulsion technique. HA was added at a concentration of 2.5 mg/ml. Microbead morphology and average diameter were assessed in acellular microbeads. MSC and ASC were seeded at a concentration of 1×10^6 cells/ml at the time of fabrication to ensure homogenous distribution of cells throughout the microbeads. Cell viability and microbead architecture was assessed at day 7. Osteogenic differentiation of MSC and ASC seeded within COL/FIB and COL/FIB/HA microbeads was monitored over 14 days of culture in media containing ascorbic acid, β-glycerophosphate, and dexamethasone. DNA, alkaline phosphatase, and calcium secretion were monitored as markers of differentiation.

Results: Fig. 1 depicts acellular COL/FIB and COL/FIB/HA microbeads. The emulsification process resulted in spheroidal three-dimensional microbeads with an average microbead diameter of 130 +/- 25 µm. Hydroxyapatite remained well-dispersed throughout the microbeads and its inclusion did not alter the diameter.

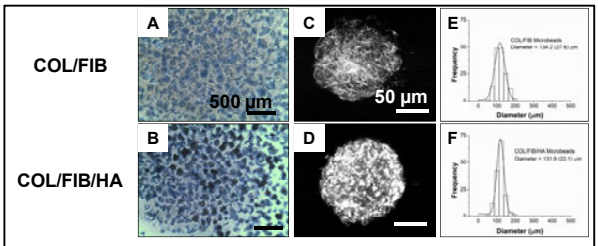

Fig. 1 – Light microscopy and confocal reflectance images of collagen/fibrin and collagen/fibrin/hydroxyapatite microbeads (A-D). Size and size distribution of microbeads (E,F).

Both COL/FIB and COL/FIB/HA microbeads supported cell viability and cell spreading of both MSC and ASC within 7 days of culture, as shown in Fig. 2, top panel. Cells also began to remodel and compact the microbeads as demonstrated through confocal reflectance microscopy (Fig. 2, bottom panel).

Fig. 3 shows the DNA, ALP, and calcium secretion data for both MSC and ASC in COL/FIB and COL/FIB/HA microbeads after 14 days in either growth or osteogenic media. In growth media, there were no significant differences in DNA content at day 7; however, the MSC COL/FIB/HA group was significantly higher compared to the other conditions at day 14. ALP activity was significantly higher at day 3 in the MSC COL/FIB/HA microbeads compared to the MSC COL/FIB microbeads. Calcium deposition markedly increased at day 14 in the MSC COL/FIB and ASC COL/FIB microbeads in osteogenic media relative to microbeads cultured in growth media.

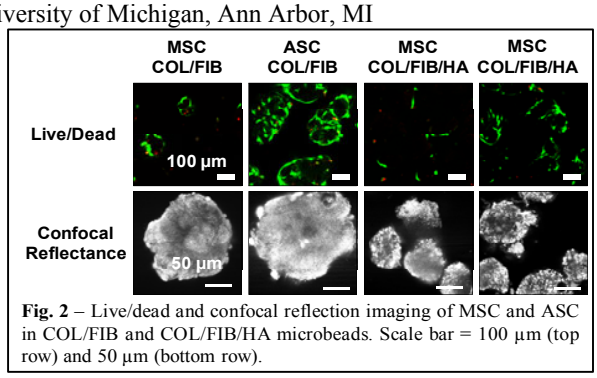

Fig. 2 – Live/dead and confocal reflection imaging of MSC and ASC in COL/FIB and COL/FIB/HA microbeads. Scale bar = 100 µm (top row) and 50 µm (bottom row).

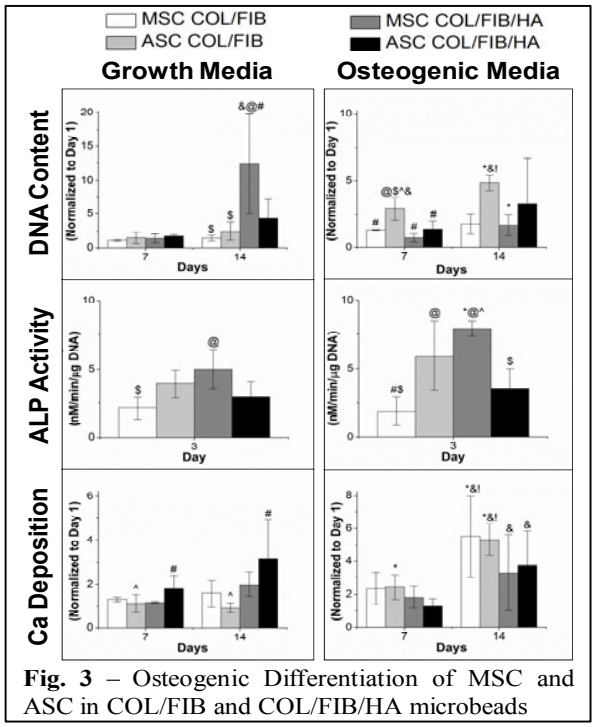

Fig. 3 – Osteogenic Differentiation of MSC and ASC in COL/FIB and COL/FIB/HA microbeads

Conclusions: These data demonstrate that adult stem cells can be encapsulated in modular protein/ceramic microenvironments. MSC and ASC stayed viable over time in culture, and the cells remodeled their matrix. After 14 days of culture in osteogenic media, both MSC and ASC mineralized COL/FIB microbeads. Such modular microbeads can be pre-differentiated towards the osteogenic phenotype, collected, and concentrated for use as a bone-filling material, potentially in a minimally invasive manner. Both MSC and ASC mineralized COL/FIB microbeads, though there was no difference between the cell types in the culture conditions tested.

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This study has relevance to modular tissue engineering for orthopaedic applications using cell-based therapy.

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