**Statement of Purpose:** Inadequate vascularization of engineered tissue constructs is a main challenge in developing clinically impactful therapies for large, complex bone defects. This problem has motivated efforts to prevascularize osteogenic constructs prior to transplantation. Mesenchymal stromal cells (MSC) are an attractive cell type given their established role in bone regeneration\(^1\) and ability to support blood vessel formation by taking on the role of a pericyte.\(^2\) However, *in vitro* culturing environments used to induce vascular and osteogenic function have proven to be incompatible.\(^3\) Thus, alternative culture systems capable of supporting vascular and osteogenic development within a unified environment are needed. In this study, we employed a modular approach in which cells were encapsulated in discrete 3D biomaterial carriers (microtissues) and precultured under desired differentiation conditions to induce appropriate MSC lineage commitment. Their modular nature enables subsequent combination to form a multi-modular construct containing multiple MSC phenotypes. This engineered construct was cultured *in vitro* and analyzed for the ability to support both vascular and osseous tissue development.

**Methods:** Modular cell-laden biomaterial carriers (microtissues) were fabricated using a water-in-oil emulsification process, as previously described.\(^4\) Vascular microtissues (VAS) were comprised of a fibrin matrix containing endothelial cells (EC) and MSC. Osteogenic microtissues (OST) were comprised of a chitosan-collagen matrix containing MSC. VAS and OST microtissues were precultured in vasculogenic (VascuLife®) and osteogenic differentiation medium, respectively. Both microtissue types were embedded in a surrounding fibrin matrix and cultured in vasculogenic medium. Vessel density, alkaline phosphatase activity and calcium mineral deposition were quantified to assess vascular and osteogenic of constructs.

**Results:** Multi-modular constructs were fabricated by embedding vascular and osteogenic microtissues within a surrounding fibrin matrix with (VAS+OST) or without (VAS+OST) preculture to induce appropriate MSC lineage commitment (Fig1A). Microtissues were evenly distributed throughout the construct and found to support the formation of an extensive vessel network by 14 days of *in vitro* culture (Fig1B). The addition of precultured microtissues were found to support a statistically higher network density within constructs compared to nonprecultured microtissues (Fig1C). When assessing osteogenic activity, constructs displayed an increase in alkaline phosphatase (ALP) levels over time regardless of microtissue preculture (Fig1D). Calcium mineral deposition was found to increase over time in constructs containing precultured microtissues compared to those with non-precultured microtissues (Fig1E).

**Conclusions:** The goal of this study was to generate an MSC-based system capable of supporting concomitant vasculogenesis and osteogenesis. We employed a microtissue approach in which cells were encapsulated in modular biomaterial carriers tailored to support either a vasculogenic or osteogenic function. This platform enabled independent preculture to induce appropriate lineage commitment and subsequent combination to form a multi-modular construct. Constructs containing precultured microtissues were found to support concomitant vessel development and osteogenic activity, thus circumventing challenges with incompatible culturing environments.

Taken together, these findings suggest a promising means for inducing and maintaining multiple MSC phenotypes within a unified system. This microtissue approach serves as an attractive platform for the development of a vascularized bone construct. Future studies will investigate chondrogenic priming as an endochondral-based strategy to better couple vascular and osteogenic components.

**References:**