Create three-dimensional orthografts using blood clots and mesenchymal stem cells *in vitro*

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INTRODUCTION: Blood clots (BCs) contain numerous biologically active factors that aid the natural healing process; accordingly, the formation of clots is foundational to the natural healing of bone and cartilage lesions [1]. Additionally, a growing body of research suggests that mesenchymal stem cells (MSCs) can promote bone and cartilage healing [2]. Therefore, this study investigated the ability of BCs loaded with MSCs to grow three-dimensional (3D) bone and cartilage graphs *in vitro*. We hypothesized that: 1) BCs loaded with MSCs would exhibit superior growth relative to controls, and 2) samples inoculated with chondrogenic or osteogenic media would exhibit molecular and morphological changes characteristic of such tissues.

METHODS: MSCs derived from the tibia and femur of 4-week old male C57BL/6J mice were isolated and cultured. Blood was collected from male C57BL/6J mice by cardiac puncture and allowed to clot without additives or in the presence of MSCs ($5x10^6$ cells/mL). After forming sable clots, samples were inoculated with either control, chondrogenic, or osteogenic media. Normoxic and hypoxic replicates were cultured at 37°C with media changed every two days; used media was stored for analysis. At 3, 7, and 21 days, samples were collected and qPCR was used to assess expression of genes associated with chondrogenesis and osteogenesis. At 28 days culture, samples were prepared for histology (H&E, Masson's Trichrome, Alcian Blue). Further, ELISA was used to characterize trends in [VEGF] and [TGFβ] within used media.

RESULTS: BCs loaded with MSCs and cultured in chondrogenic or osteogenic media formed 3D sphere-shaped structures (Figure 1) that, upon histological examination, were found to have undergone morphological changes consistent with chondrogenesis and osteogenesis, respectively. Additionally, we noted increased release of tissue-specific factors in the media of such samples. For chondrogenic samples, preliminary data suggests that samples cultured in hypoxic conditions released superior [TGFβ], relative to normoxic samples (Figure 2). Ongoing experiments are examining changes in expression of chondrogenic and osteogenic gene products.

DISCUSSION: These results suggest that BCs serve as biologically active scaffolds that facilitate tissue growth. Further, the amount and morphology of grown tissue appears to respond to local, tissue-specific factors (i.e., those within the growth media). This appears to be related to the increased amounts of TGFβ and VEGF that are released by BC/MSC preparations. These *in vitro* findings may partially explain the mechanistic basis by which application of autologous BCs promote improved healing of cartilage and bone defects *in vivo*.

SIGNIFICANCE/CLINICAL RELEVANCE: Autologous BCs are an appealing therapeutic option for the repair of cartilage and bone abnormalities because they can be made quickly, readily, and affordably. The effectiveness of the 3D model can be utilized to guide additional research into the mechanisms through which MSC-loaded BCs encourage bone and cartilage repairs.

REFERENCES:

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FIGURES:

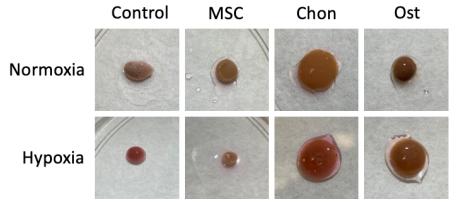

Figure 1. Gross morphology of BCs after 8 weeks culture. Width of each box is 2 cm.

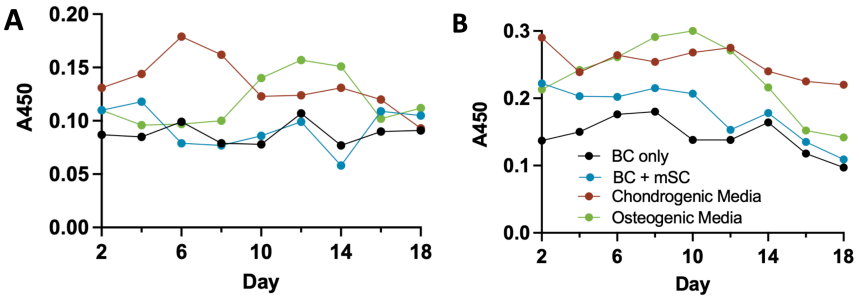

Figure 2. [TGFβ] released from BCs *in vitro*. Samples cultured in normoxic conditions (A) showed inferior concentrations than hypoxic samples (B).