Mesenchymal Progenitor Cells Derived From Traumatized Muscle Secrete Factors That Regulate Angiogenesis

INTRODUCTION
Regulation of angiogenesis, the process of new blood vessel growth, is critical to the engineering of vascularized tissues. For example, in order for new blood vessels to sprout during the early stages of angiogenesis, the existent vascular matrix is remodeled by secreted proteases such as plasmin and matrix metalloproteinases (MMPs). Conversely, at the end of angiogenesis, newly formed vessels are stabilized by protease inhibitors such as tissue inhibitor of metalloproteinases (TIMPs). Mesenchymal stem cells (MSCs) are attractive candidates for inclusion in vascular tissue engineering constructs due to their secretion of both proteases and protease inhibitors. We have recently identified a population of mesenchymal progenitor cells (MPCs) that can be harvested from traumatized muscle tissues and exhibit several advantages, including higher isolation efficiencies, over MSCs that might make them logistically preferred in clinical applications. However, the contributions of MPCs to the proteolytic microenvironment have never been characterized. The goal of this study is to compare the vascular matrix-modifying factors secreted by MSCs and MPCs in the context of angiogenesis regulation.

MATERIALS AND METHODS
Cell Culture: MSCs were isolated from human hip trabecular bone and MPCs were isolated from human muscle samples as tissue culture plastic adherent cell populations. MSCs and MPCs were expanded in high-glucose DMEM containing 10% FBS and passaged at 70-80% confluency. All experiments were performed with passage 3-5 primary cells. The microvascular endothelial cell (EC) line HMEC-1 was expanded in Clonetics® EGM-2-MV™ medium.

Conditioned Medium: MSCs and MPCs from 3 patients each were seeded at 1.0x10⁶ – 1.25x10⁷ cells/cm² in 150 cm² dishes, cultured until sub-confluent (typically 3 days) in expansion medium and then incubated with serum-free (SF) phenol red-free (PF) media (15 ml per dish) for 1, 2, or 4 days. The media were then collected and concentrated by centrifugation using spin columns (Amicon Ultra-15 3 kDa NMWL; typical concentration yields: 20-40x by volume) to generate conditioned media (CM). CM aliquots were analyzed by SDS-polyacrylamide gel electrophoresis SDS-PAGE and western blotting for plasminogen.

Zymography: Gelatin zymography was performed using 8% SDS-PAGE containing 1 mg/ml gelatin. 15% gels containing 2.5 mg/ml and 160 ng/ml MMP-9 were used for reverse zymography. Day 1, 2, and 8 MSC-CM and MPC-CM samples were resuspended to 5X in water, mixed with zymogram sample buffer and separated with SDS-PAGE at 4°C. The gels were then equilibrated with zymogram retenation buffer and incubated with zymogram development buffer overnight at 37°C. Bands were visualized by staining with Simply Blue Safe Stain. Representative scans of 3 patients tested for each cell type are presented.

RESULTS
MSCs and MPCs isolated from 3 patients express factors that regulate extracellular matrix remodeling, a critical process in angiogenesis. Western blot (Figure 1A) and gelatin zymography (Figure 1B) analysis of cells cultured under control conditions showed that MSCs and MPCs secrete the proteases plasmin, MMP-2, and MMP-9. MSCs and MPCs secreted similar levels of plasmin that remained constant over 4 days of culture. MSC-secretion of MMP-2 and MMP-9 began low and increased during culture. MPCs, on the other hand, exhibited higher MMP-2 and MMP-9 levels for early time points that remained consistent over the culture period. Reverse zymography analysis of MSC- and MPC-CM (Figure 1B) showed that both cell types secrete TIMP-1 and TIMP-2. The relative levels between the TIMPs were comparable, with more TIMP-1 being secreted than TIMP-2 for all time points tested. MSC secretion of both TIMP-1 and TIMP-2 remained relatively constant over 4 days of culture, while MPC TIMP-secretion was delayed, not beginning until day 2. The MSC-CM from days 1 and 4 induced higher EC monolayer permeabilities than MPC-CM from the same time points (Figure 1C).

DISCUSSION
Both MSCs and MPCs secrete proteases and protease inhibitors previously shown to be important in regulating the vascular microenvironment. However, certain key differences in expression profiles between the two cell types may lend them to different applications in vascular tissue engineering. MPC-MMP secretion begins early during culture, while MPC-TIMP secretion is delayed. This is in contrast to MSCs, which exhibit the reverse trend. The protease/inhibition secretion time-course of MPCs is more conducive to angiogenesis, which requires early matrix degradation and later matrix stabilization. These observations are supported by vascular permeability assays; EC monolayers incubated with MPC-CM are less permeable than those incubated with MSC-CM, indicating that MPC-CM stabilizes EC structures. These differences between the two cell types suggest MPCs generate a microenvironment supportive of early vessel formation and later stabilization. Future research will focus on further characterizing the effects of MPC-secreted factors on angiogenesis.

REFERENCES:

ACKNOWLEDGEMENTS:
Supported by a grant from the Military Amputee Research Program at WRAMC (POS-A011) and by the Intramural Research Program at the NIH, NIAMS (Z01 AR41131).