The Angiogenic and Osteogenic Effects of Cyclic Tensile Strain on a Co-culture of Human MSCs and HUVECs

Andrew J. Steward[1], Jacqueline H. Cole[1], Frances S. Ligler[1], Elizabeth G. Loboa[1,2]
[1] Joint Department of Biomedical Engineering at UNC-Chapel Hill and NC State University
[2] Department of Materials Science and Engineering at NC State University

Disclosures: Andrew J. Steward (None), Jacqueline H. Cole (None), Frances S. Ligler (None), Elizabeth G. Loboa (None)

INTRODUCTION: Many current bone tissue engineering strategies neglect the fact that development and maintenance of a vascular network is critical for bone growth and homeostasis, limiting the potential size and success of tissue engineered constructs.[1] Strategies that promote and enhance vascular activity in constructs pre-implantation are critical for clinical success of tissue engineered bone constructs.[2] As the need for vascularized constructs has grown, the number of studies investigating the interactions between mesenchymal stem cells (MSCs) and endothelial cells (ECs) has expanded. We have recently found and reported that angiogenic factors such as VEGF are increased in MSCs exposed to 10% cyclic tensile strain.[3] However, the role of the physiologic mechanical environment on MSC-EC crosstalk has yet to be investigated. Therefore, the objective of this study was to expose a co-culture of MSCs and ECs to 10% cyclic tensile strain to examine the role of this mechanical stimulus on MSC-EC behavior. We hypothesized that paracrine signaling from ECs would stimulate osteogenesis of MSCs, and the application of 10% cyclic tensile strain of ECs would further enhance this anabolic signal.

METHODS: Human bone marrow-derived MSCs were isolated (19 year old female) and expanded in growth medium (GM=αMEM, 10% FBS, P/S). Human umbilical vein endothelial cells (HUVECs) (Lonza) were expanded in endothelial growth medium (EGM=GM, 30 μg/ml endothelial complete growth supplement (ECGS), 100 μg/ml heparin). MSCs and HUVECs were either mono or co-cultured at a 1:1 ratio (2 x10^4 MSCs and 2 x10^4 HUVECs per well) in collagen type I coated BioFlex® culture plates and exposed to 10% cyclic tensile strain using the Flexcell® Tension Plus™ System at 1 Hz for 4 h/d for 2 weeks. MSC mono- and MSC-HUVEC co-cultures were grown in a mixed osteo/angiogenic media (EGM, 50 mM ascorbic acid, 100 μM dexamethasone, and 1 M glycerophosphate). Samples were stained for CD31, VE-Cdh, and Alizarin Red. Calcium accretion and DNA were evaluated on day 14 using the Calcium Liquicolor and Hoechst assays, respectively. Comparisons between samples exposed to 10% cyclic tensile strain and unloaded, and between mono and co-cultured samples, were analyzed using a 2-way ANOVA, with a $p < 0.05$ considered significant.

RESULTS: While co-culture of MSCs with ECs did not significantly alter calcium accretion (Fig. 1A), a slight increase was observed both quantitatively and qualitatively (Fig. 1A,B). Exposure to 10% cyclic tensile strain significantly enhanced osteogenesis in both mono- and co-culture (Fig. 1A). The combination of co-culture and cyclic tensile strain appeared to act synergistically resulting in an even greater increase in calcium accretion (Fig. 1A). Consistent with the quantitative calcium accretion data (Fig. 1A), both cyclic tensile strain and MSC-HUVEC co-culture resulted in enhanced mineral deposition (Fig. 1B). Neither MSC-HUVEC co-culture nor 10% cyclic tensile strain appeared to affect CD-31 or VE-Cdh staining (Fig. 1C).

DISCUSSION: Co-culture of MSCs with HUVECs enhanced osteogenesis as quantified by cell-mediated calcium accretion, suggesting that interactions with ECs may improve the osteogenic potential of MSCs. The application of 10% cyclic tensile strain was found to enhance osteogenesis of both MSC mono and MSC-HUVEC co-cultures. The results of the mono-culture are consistent with previous studies that indicated that 10% cyclic tensile strain enhances osteogenesis of MSCs.[3,4] The response of the MSC-HUVEC co-cultures to 10% cyclic tensile strain was significantly greater than the response of MSCs alone, suggesting that co-culture of MSCs and ECs and exposure to 10% cyclic tensile strain synergistically enhanced calcium accretion and mineral deposition. Endothelial markers CD-31 and VE-Cdh did not appear to be affected by either co-culture or mechanical stimulation, suggesting that the endothelial phenotype remained stable but was unresponsive to the stimuli evaluated in this study. Overall, co-culture of MSCs with HUVECs and exposure to 10% cyclic tensile strain synergistically enhanced osteogenesis while maintaining endothelial phenotypic stability. Future studies should focus on the specific paracrine signals stimulated by co-culture and 10% cyclic tensile strain in order to better understand the underlying mechanisms regulating MSC-EC interactions and improve tissue engineering and regenerative medicine strategies.

SIGNIFICANCE: This is the first study to investigate the role of cyclic tensile strain on the complex interplay between MSCs and HUVECs in co-culture. The results of this study provide key insights into the synergistic effects of co-culture and cyclic tensile strain on both angiogenesis and osteogenesis. Understanding the cross-talk between stem cells and endothelial cells, and mechanobiological factors affecting such cross-talk, will help to enhance current strategies for creating vascularized tissues in tissue engineering and regenerative medicine.

REFERENCES:

1. Nguyen, L. H. *et al.* Tissue Eng. Part B Rev. 18, 363–382 (2012).
2. Kanczler, J.M. & Oreffo, R.O.C. Eur. Cell. Mater. 15, 100–114 (2008).
3. Charoenpanich, A. *et al.* Tissue Eng. Part A 20, 67–78 (2013).
4. Sumanasinghe, R. D., *et al.* Tissue Eng. 12, 3459–3465 (2006).

ACKNOWLEDGEMENTS: This study was supported by a William R. Kenan, Jr. Institute for Engineering, Technology and Science Pilot Research Grant, NIH/NIBIB 1R03EB008790 (EGL), and NSF/CBET 1133427 (EGL).

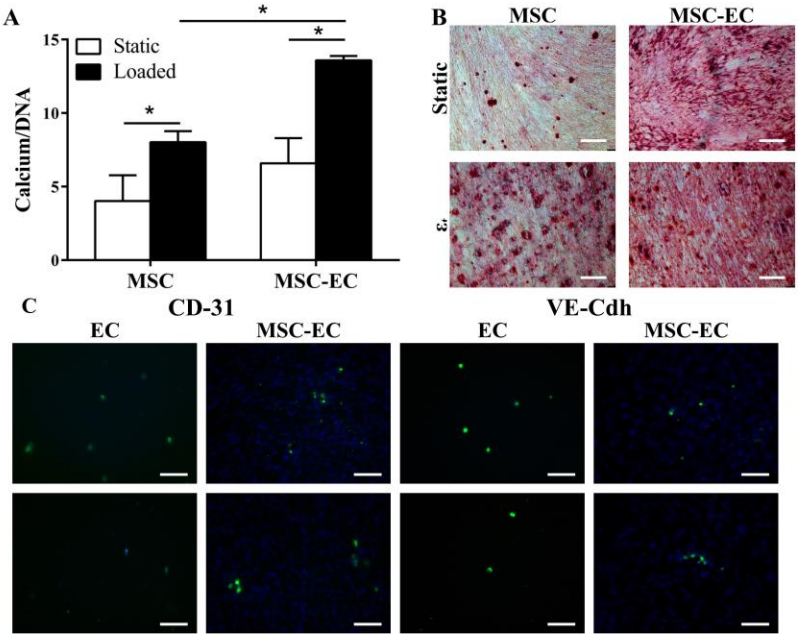

Figure 1. (**A**) The effects of 10% cyclic tensile strain (loaded) on calcium/DNA accreted by mesenchymal stem cells (MSC) mono-culture or co-culture of MSC and human umbilical vein endothelial cells (MSC-EC). Representative images of (**B**) Alizarin Red and (**C**) CD-31, and VE-Cdh of MSC mono- and MSC-HUVEC co-cultures after culture for 14 days under either static (unloaded) or mechanically loaded (10% cyclic tensile strain, 1 Hz, 4 hours/day) conditions. *$p < 0.05$. Scale bars=100 μm.