A Hypoxic Environment Modulates Mesenchymal Stem Cell Function and the Exerted Effects on Macrophages

Laura M. Ricles[1], Eunna Chung[1], Laura J. Suggs[1]
[1]Department of Biomedical Engineering, The University of Texas at Austin, Austin, TX, U.S.A., 78712-0238

Statement of Purpose: Cardiovascular and ischemic diseases are a growing health concern in developed countries. Stem cell therapy, and in particular mesenchymal stem cells (MSCs), are being investigated as an alternative therapy for ischemic diseases. Previously we have demonstrated that an insoluble PEGylated fibrin biomatrix can promote MSC tubulogenesis without the addition of growth factors, and potentially lead to increased neovascularization *in vivo.* [1] However, following delivery, the cells are exposed to a hypoxic environment, resulting in modifications in cellular function. The modulation of stem cell behavior also has influences on other cell types within the body, such as macrophages, which have been shown to have key roles in wound healing. The upregulated secretion of certain soluble factors by stem cells within a hypoxic environment can lead to increased macrophage migration to the hypoxic site and modulation of the macrophage phenotype [2]. The current study investigated the influence of an *in vitro* hypoxic environment on MSC behavior and the impact of MSC conditioned media on macrophages.

Methods: Human MSCs were cultured in serum free media under normoxic or hypoxic (2% O_2) conditions for 48 hours. The media was collected every 24 hours and replaced with serum free media. The cells were collected, RNA was extracted and converted to cDNA, and real-time PCR was performed to analyze gene expression. Protein was also isolated from the cells and relative protein expression was analyzed using western blotting. Cell number following culturing was quantified using an MTS assay. The protein expression of HIF-1 alpha was quantified using ELISA analysis following 6 hour culture. The expression of angiogenic and cytokine factors within the culture media was performed by Quansys Biosciences. The influence of MSC conditioned media on hU937 macrophages was analyzed. hU937 cell proliferation was quantified using an MTS assay. Also, a Boyden migration assay was used to assess the chemotactic effect of the conditioned media.

Results: MSC Response to Hypoxia: Following 6 hour culture in a hypoxic environment, MSCs exhibited a stress response as evidenced by the upregulation of HIF-1 alpha protein expression (Figure 1a). In addition, western blot analysis showed that HSP70 protein expression was upregulated following 48 hour hypoxic culturing, further supporting the stress responsiveness of MSCs in a hypoxic environment. MSC proliferation was significantly increased for cells undergoing hypoxia compared to normoxia cells (Figure 1b), which can be attributed to either extended proliferation capacity or decreased population doubling time [3]. Gene analysis demonstrated that a hypoxic environment promoted MSC phenotype to transition from more pericyte-like under normoxic conditions to endothelial-like and angiogenic promoting under hypoxic conditions, with the downregulation of SMA and the upregulation of PECAM and MMP2 following hypoxic culturing (Figure 1c). Protein analysis follows similar trends as the gene analysis.

Conditioned Media Culturing of Macrophages: Analysis of the conditioned media collected from hypoxic and normoxic MSC cultures showed enhanced secretion of FGF and IL-8 for MSCs under hypoxia. hU937 macrophages were cultured with conditioned media collected from normoxic and hypoxic MSC cultures. Following 48 hour culture, macrophages exhibited an increase in cell proliferation for hypoxic conditioned media compared to serum free and normoxic conditioned media (Figure 1d). In addition, cell migration was increased for hypoxic conditioned media groups.

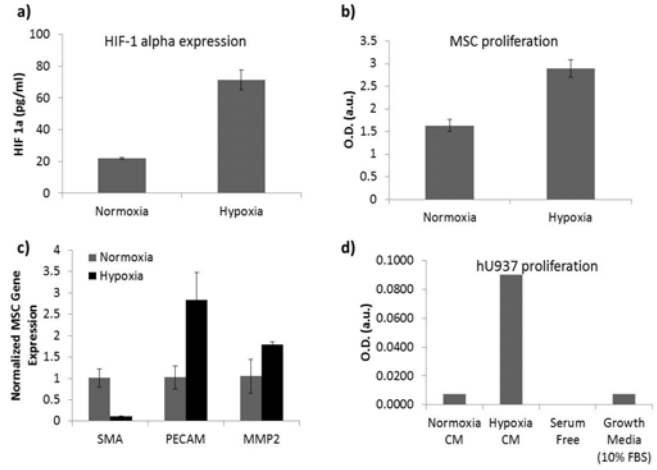

Figure 1. The effect of a hypoxic environment on a) HIF-1 alpha expression, b) proliferation, c) and gene expression of MSCs. d) Influence of MSC conditioned media on hU937 proliferation.

Conclusions: This study analyzed the influence of a hypoxic environment on MSC function and the resulting influence of MSCs on macrophages. MSCs undergo a stress response accompanied by increases in cell proliferation, phenotypic changes, and changes in secretion of soluble factors. In addition, hypoxic conditioning of MSCs influences macrophages and could modulate the cells and promote a more conducive environment for wound healing. Future work will involve analyzing the hypoxic environment of stem cells cultured in 3D PEGylated fibrin gels and the resulting effects on macrophages. The results of the current study will be used in combination with an *in vivo* ischemia model and will help to elucidate the mechanisms responsible for the enhanced angiogenic effect of MSCs seeded within PEGylated fibrin gels and delivered into a hypoxic environment.

References:

1. Zhang G. Act Biomater. 2010;6:3395-3403.
2. Eggenhofer E. Transplantation Research. 2012;1:1-5.
3. Das R. Tissue Eng B. 2010;16:159-168.