

TRAUMA/CRITICAL CARE 1: SIGNALING AND GROWTH FACTORS

32.1. Effect of Burn Injury on Mesenchymal Stem Cell Niches: the Mechanism and Possible Treatment for Heterotopic Ossification. B. Levi, J. Lisiecki, J. Rinkinen, J. Peterson, S. Deshpande, J. Bayliss, M. Hemmilla, C. Xi, G. Su, S. R. Buchman, P. S. Cederna, S. C. Wang; University of Michigan, Ann Arbor, Michigan

Introduction: Heterotopic ossification (HO), or the abnormal development of bone in soft tissue locations, can be a clinically devastating sequela of burn injury often leading to restricted joint mobility, severe pain, and nerve entrapment. An accepted mechanism for HO proposes that stem cell-like progenitors become aberrantly activated to form bone. We hypothesize that the massive inflammatory response to burn injury enhances the osteogenic capacity of human and mouse mesenchymal stem cells (MSC). Furthermore, we hypothesize that this osteogenic potential can be mitigated through Adenosine triphosphate (ATP) inhibition at the burn site. **Methods:** Human MSCs cells were harvested from adipose tissues of burn patients and age matched controls. Mouse MSCs (Bone marrow derived and adipose derived) were harvested 2, 6, and 24 hours after burn injury, burn injury + ATP inhibitor application or non-burn control (n=4 per group). Subsequently, osteogenic capacity was assessed by gene expression (qRT PCR), protein expression (western blot) and standard *in vitro* osteogenic differentiation assays. *In vivo* heterotopic bone formation was assessed using an achilles tenotomy model. *In vivo* osteogenic signaling was assessed by immunohistochemistry and heterotopic bone formation was quantified by histomorphometry, Raman spectroscopy and microCT. **Results:** Human ASCs from burn patients demonstrated increased osteogenic differentiation and expression of the early osteogenic marker Runt-related transcription factor-2 (RUNX2), the late osteogenic marker osteocalcin (OCN), and BMP-2 ligand (p<0.05). Similarly, burn injury resulted in a striking increase in osteogenic differentiation and osteogenic gene expression among mouse MSCs at all time points which was mitigated by ATP inhibition at the burn site. In both human and mouse cell lines, increased osteogenic differentiation correlated with increased BMP-2 signaling as demonstrated by phosphorylated smad 1/5 expression by Western Blot analysis. In our achilles tenotomy model, burn injury also enhanced *in vivo* osteogenic signaling by histology as well as increased bone volume and bone mineral content by Raman Spectroscopy and microCT (Fig, *p<0.05). This HO formation was blunted in mice after ATP inhibition at the burn site. **Conclusions:** We demonstrate that burn injury enhances the osteogenic capacity of MSCs *in vitro* and heterotopic bone formation *in vivo*. The mech-

anism appears to be due to up-regulation of BMP-2 and this can be mitigated by blocking ATP at the burn site both *in vitro* and *in vivo*. We establish a potential role for burn injury in modulating heterotopic bone formation and demonstrate that direct manipulation of inflammation at the burn site may have therapeutic utility in treatment regimens designed to prevent and remediate HO.

32.2. Effect of An NF- κ B Inhibitor on the Cell Population in the Fibroblast-Populated Collagen Matrix. D. A. Doyle,¹ J. Chao,¹ D. Heimann,^{1,2} T. Pea,³ C. Hansen,^{1,2} M. A. Carlson,^{1,2}; ¹University of Nebraska Medical Center, Omaha, NE; ²Veterans Affairs Medical Center, Omaha, NE; ³University of Nebraska Lincoln, Lincoln, NE

Introduction: Detachment of the fibroblast-populated collagen matrix (FPCM) from its substratum induces apoptosis and inhibits the cell cycle, thus reducing matrix cell number. Microarray and immunoblot data suggested that NF- κ B activation may participate in cell population regression after matrix detachment. We hypothesized that addition of a small molecule inhibitor of NF- κ B to the system would prevent the decrease in matrix cell number after detachment of the FPCM from its substratum. **Methods:** Two models of the 3D collagen matrix (1.5 mg/mL; 0.2 mL volume) populated with human foreskin fibroblasts (200,00 cell per matrix) were used to test the effects of NF- κ B inhibition on cell matrix number. Model 1 involved adding N-p-tosyl-L-phenylalanine chloromethyl ketone (TPCK, a known inhibitor of NF- κ B activation) directly at the time of matrix set-up, then allowing each matrix to incubate for 24 hr prior to physical detachment of the matrix from the culture dish. The matrices were harvested 72 hr after detachment, and cell counting was performed with an automated counter. In Model 2, matrices were incubated for 24 hr, detached, and then TPCK was added into the medium (5% FBS in DMEM), followed 72 hr later by cell counting. **Results:** The results of the cell counting (normalized in each experiment to the untreated attached value) for attached (Att) vs. detached (Det) matrices in both models are illustrated in the Figure. Each bar = mean \pm SD of triplicate experiments; *p < 0.05 compared to Att (Mann-Whitney U). TPCK treatment reversed the effect of matrix detachment on cell counts for at least one concentration in both models. There was no evidence of TPCK toxicity with respect to counts in neither the Att nor Det matrices (p > 0.15, Kruskal Wallis). **Conclusions:** FPCM detachment from substratum induced cell population regression, as previously shown. FPCM treatment with the NF- κ B inhibitor TPCK, whether at the time of matrix set-up (Model 1) or just prior to detachment (Model 2), prevented the decrease in cell number that occurred in the matrix after detachment from its substratum. This data supports our hypothesis, indicating that NF- κ B activation may participate in the regression of matrix cell population after detachment of the FPCM.

