Introduction: Traumatic spinal injury always involves complex tissue damage to both the spinal and vertebral column. Thus, effective therapies require both neurogenic repair and osteogenesis. To this end, exogenous-mesenchymal stem cell (MSC) therapy has been investigated, based on its anti-inflammatory and dual-differentiation effects on fracture healing and neuronal regeneration. Additionally, several biologic protein therapies have shown promise for this purpose. Previously, we demonstrated that systematic parathyroid hormone (PTH) therapy significantly increased exogenous MSC homing to femoral fracture callus. Prior studies have shown beneficial effects of epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), and human mesenchymal stem cells (hMSCs) therapy based on their known neurogenic and immunomodulatory effects. Here we tested the hypothesis that PTH therapy-induced angiogenesis and osteogenesis synergizes with EGF, bFGF and hMSCs neurogenic therapy during healing of a hemisection spinal cord lesion in mice.

Methods: In vitro gene expression of GFAP, beta 3-tubulin, nestin and MAP2 was assessed in primary bone marrow derived human MSCs (Lonza Group Ltd) by RT-PCR and Western-Blot following culture with: 1) vehicle, 2) PTH, 3) EGF/bFGF and 4) PTH+EGF/bFGF on days1, 3, 5 and 7 (n=3). The spinal cord injury model of C57BL/6 female mice received a T11 spinal cord hemisection lesion (SCI) and its T11 laminectomy as described previously1, and 106 hMSCs were transplanted locally into the lesion site and randomized into the following treatment Groups: 1) hMSC, 2) hMSC+PTH, 3) hMSC +PTH+EGF/bFGF and for non-hMSCs treatment groups: 1) vehicle, 2) PTH, 3) PTH+EGF/bFGF (n>4) for 4-5 weeks. Then spines were harvested after cardiac perfusion with a vascular dye (MV-122) to access angiogenesis and osteogenesis by micro-CT, followed by immunohistochemistry (IHC) for MAP2, GFAP and beta-3 tubulin (primary antibody 1:500 dilution) to assess neuronal regeneration.

Results: 1) In vitro, PTH synergizes with EGF/bFGF to significantly enhance gfap (day3), beta-3 tubulin (day5) and map2 (day 3,5) expression in hMSCs 3-6 fold in a time dependent manner (Figure 1A), and this increase at the protein level was confirmed by Western-Blot (Figure 1B).
2) In vivo, the combined triple biologic and hMSC therapy significantly enhanced angiogenesis in spinal cord tissue at the injury site, as the total volume of these microvessels (Ø<0.126mm) were increased 120% vs. vehicle (p<0.05) (Figure 2A), which was proportional to the 20-30% increase in new trabecular
bone formation and its connectivity density (p<0.05) (Figure 2B), and was associated with increased neurogenesis determined by IHC (Figure 3).

**Discussion:** Combination biologic and MSC therapy synergizes to mediate hemisection repair via increased angiogenesis, osteogenesis and neuronal regeneration. Formal studies are warranted to confirm these synergistic effects and elucidate the underlining mechanism of action.

**Significance:** PTH therapy-induced angiogenesis and osteogenesis synergizes with EGF, bFGF and hMSCs neurogenic therapy during healing of a hemisection spinal cord lesion.

**Figure 1.** Synergistic effect of biologic combination of PTH, EGF & bFGF on hMSCs neurogenic differentiation were checked using mRNA and protein that isolated from different treatment groups (p<0.01 vs. control group).
Figure 2. Total volume and proportional histogram distribution of vascular within SCI site, and tubercular bone structure of T11 vertebra body were accessed by high-resolution micro-CT at 5 weeks post injury. (p<0.05, vs. control)
Figure 3. IHC was performed on paraffin embedded injury spinal cord tissue section to confirm the neurogenic markers. (200x)