Characterization of Human Skeletal Stem Cells in Closed and Open Tibia Fractures: A Single Center Pilot Study

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INTRODUCTION: Tibial shaft fractures are the most common long bone fractures and carry a significant risk of nonunion, particularly in open injuries. This increased risk has been attributed to heightened activation of damage-associated molecular pathways, cellular senescence, and alterations in the fracture milieu. However, most supporting evidence arises from animal studies under controlled conditions, and the cellular and biochemical environment of human traumatic fractures remains poorly understood. While mechanical and patient-related factors have been associated with impaired healing, the biological mechanisms are not well defined. Human skeletal stem cells (hSSCs) play a critical role in bone regeneration and may provide insight into differential healing responses. This study aimed to characterize hSSC recruitment and functional capacity in open versus closed tibial fractures.

METHODS: Following institutional review board approval and informed consent, a prospective pilot study of patients undergoing acute tibial shaft fixation at a Level 1 trauma center was conducted. During intramedullary nailing, reamings were collected for analysis. hSSCs were isolated *via* flow cytometry. Functional assays included colony-forming unit (CFU) counts and *in vitro* osteogenic differentiation *via* Alizarin Red staining. $CD146^+$ osteostromal cells were quantified, and serum alkaline phosphatase (ALP), IL-6, and HbA1c levels were analyzed. Statistical comparisons were made between groups, and outliers were identified using the ROUT method (Q=5%).

RESULTS SECTION: 19 patients (6 females, 13 males, mean age = 40.5) with isolated tibial shaft fractures (8 closed, 11 open) treated with intramedullary nailing were included. hSSC frequency was significantly lower in open fractures compared to closed fractures after outlier exclusion (2.75% ± 1.67 vs. 5.64% ± 5.80, p=0.032), suggesting reduced early recruitment. However, no significant differences were observed in CFU capacity (0.0078 ± 0.0071 vs. 0.0156 ± 0.0117, p=0.221) or osteogenic differentiation (1.24 ± 0.22 vs. 1.52 ± 0.85, p=0.419). $CD146^+$ cell levels and serum markers were similar between groups. These results are displayed in Figure 1. ALP levels correlated strongly with $CD146^+$ cell abundance (Figure 2) in closed fractures (ρ=0.80, p=0.02) but not with hSSC levels.

DISCUSSION: Open tibial fractures demonstrate reduced early hSSC recruitment compared to closed fractures in the acute period of fracture healing, while *in vitro* stem cell function appears preserved. These findings support a model in which impaired healing in open fractures may result from reduced hSSC recruitment rather than dysfunction. Limitations of this study include a small cohort and limited follow-up duration. Larger studies with long-term clinical follow-up are warranted to validate these results and explore therapeutic strategies targeting the hSSC niche to enhance fracture healing in high-risk populations.

SIGNIFICANCE/CLINICAL RELEVANCE: This pilot study demonstrates that human skeletal stem cells are significantly less abundant in open tibial fractures compared to closed fractures, suggesting a potential cellular basis for the higher nonunion rates observed in open injuries. These findings underscore the importance of defining fracture-site cell populations as a foundation for future hSSC-targeted diagnostics and regenerative therapies.

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FIGURES:

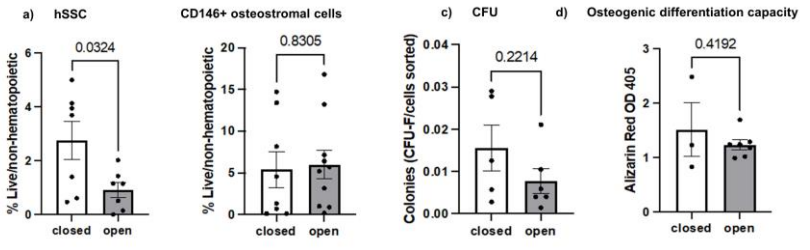

Figure 1

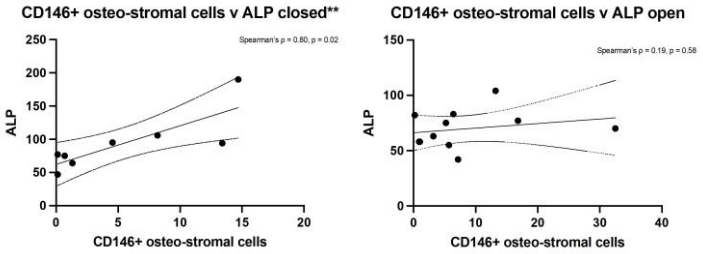

Figure 2