Analysis of Murine Bone Marrow Autografts Iliac Crest versus Femoral Shaft using Mass Cytometry

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INTRODUCTION: Fracture healing is a complex dynamic process; delayed union or non-union may occur due to various factors, including poor vascularization or innervation, fracture instability, and infection. To solve this demanding challenge, bone autografts are implemented as a promising surgical intervention to enhance fracture healing. Iliac crest is the most common site for cancellous bone grafts, owing to its easy accessibility, enriched mesenchymal stem cells (MSCs) and biomechanical stability and large volume [1]. Autologous bone marrow cell transplantation demonstrated augmented bone formations in lower extremities fracture cases with limited postoperative complications [2]. Moreover, the collection of osteogenic MSCs from various sites such as iliac crest, femoral shaft, and vertebral body, has been widely explored. While studies have compared the clinical outcomes of different autografts [3], the distinctive attributes of cell subtypes and functionalities from diverse bone marrow sources remain unknown. To bridge this knowledge gap, we present a comparative investigation of murine bone marrow from iliac crest (IC) and femoral shafts (FS) employing high-dimensional Cytometry by Time-of-Flight mass spectrometry (CyTOF).

METHODS: The experimental design received approval from the Institutional Administration Panel for Laboratory Animal Care at Stanford University. Bone marrow cells were collected from IC and FS of BALB/cByJ mice (8-12 weeks old, n = 8 per each group) respectively under sterile conditions. Briefly, the bone marrow was flushed and filtered by injecting basal medium (RPMI Medium 1640 supplemented with 10% fetal bovine serum, 1% Antibiotic-Antimycotic). Cells were spun down and resuspended in ice-cold red blood cell lysis buffer, followed by the addition of 20 mL/tube basal medium. Mass Cytometry was employed, the harvested samples were dissociated, fixed, barcoded, permeabilized, stained with the metal isotope-tagged antibody cocktail, and re-suspended in MilliQ water before CyTOF analysis (Fig 1A). A 40-antibody panel (Fig 1B) was used to identify certain cell populations and signaling pathways. Antibodies were purchased pre-conjugated from Fluidigm or were conjugated in-house to purified, carrier-free stocks from Biolegend and Abcam. Data analysis utilized FlowJo v10.6 (mass cytometry settings) for cell subset-specific gating and cloud-based Cytobank for multiparametric analysis, including viSNE.

RESULTS: Standardized gating strategies were applied across all samples to exclude cell debris, doublets, dead cells, and red blood cells (RBCs). viSNE analysis demonstrated distinctive major cell populations, with their phenotypic markers indicating distribution patterns. Comparison of major cell populations as a percentage of total non-RBCs showed variations between IC and FS samples (Fig 2A, B). Notably, the presence of granulocytes ($CD45^+$, $Ly6G^+$) was significantly different, constituting 49.0% in IC and 55.2% in FS, with a majority of $CD44^+$ cell subpopulation in granulocytes within FS (Fig 2C). Furthermore, the MSC population was rare (0.6%), and muscle satellite cells accounted for 2.5% in IC. FS has significantly higher enrichments, 1.5% and 16.8% respectively vs. non-leukocytes ($CD45^-$, Fig 2D, E). Regarding the leukocytes ($CD45^+$), the major difference was found in macrophages. Among general macrophages ($CD11b^+$, $F4/80^+$) which account for 7.0% in IC and 8.5% in FS vs. mononuclear cells ($CD45^+$, $Ly6G^-$), the polarized subtypes are significantly different. IC (0.8%) contains less M2 macrophages ($CD206^+$) than FS (3.9%) over macrophages. M0 macrophages accounted for major subtypes in both IC (88.9%) and FS (83.4%) (Fig 2F-H).

DISCUSSION: Our study compared the cell composition between two typical autografts in mice. Utilizing high-dimensional CyTOF, cell profiles within distinct bone marrow sites were elucidated. For example, bone marrow from FS contains 2 times more MSCs, 5 times more muscle satellite cells, and interestingly 10 times more anti-inflammatory M2 macrophages than those in IC samples. These differences indicated that femoral bone marrow had better cell functionality with higher bone regeneration potential and immunological attributes. The forthcoming CITRUS analysis will be performed to identify further properties of these cells.

CLINICAL RELEVANCE: This investigation holds substantial clinical implications, furnishing insights into the selection of diverse bone marrow sources for autograft transplantation during surgical interventions. A comprehensive understanding of cell composition and functionalities, particularly regarding osteogenesis and immune properties, stands to advance personalized treatment approaches for fracture patients with complex biological microenvironments.

REFERENCES: [1]Raghuram et al. Semin Plast Surg. 2019. [2]Verboket et al. Eur J Trauma Emerg Surg. 2018. [3]Palombella et al. Stem Cells Int. 2019.

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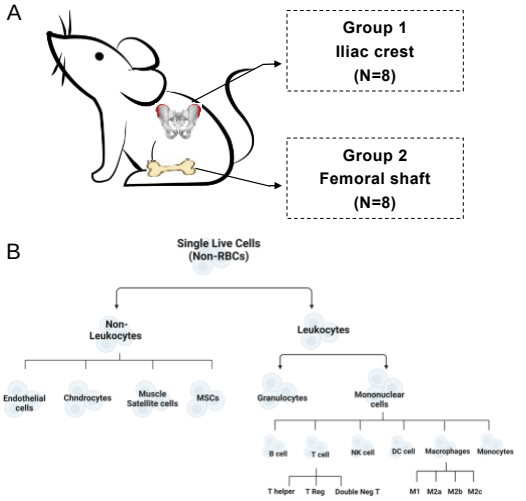

Fig 1. Experimental design of different autografts comparisons and antibody panels.

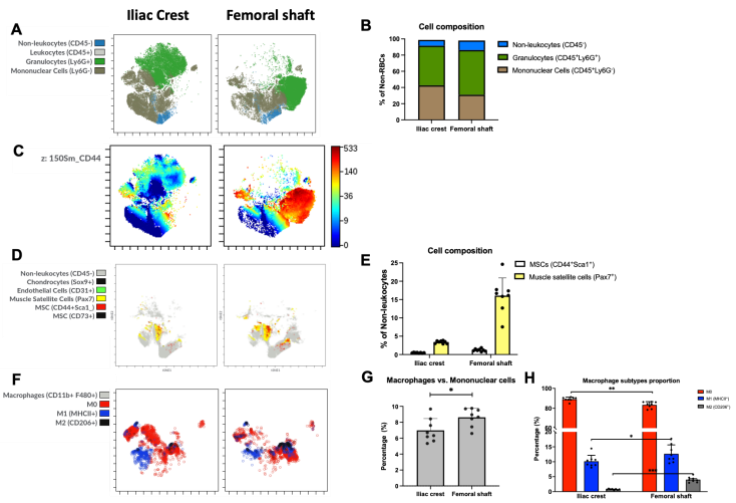

Fig 2. Multiparametric analysis of cell compositions.