Stromal Cell-Derived Factor-1 and Monocyte Chemotactic Protein-3 Improve Recruitment of Osteogenic Cells into Sites of Musculoskeletal Repair

Introduction
Clinical success in settings of bone repair or regeneration requires the function of osteogenic progenitors. Traditional methods to improve bone repair (e.g., implantation of a scaffold matrix such as allograft) rely upon the local intrinsic population of osteogenic progenitors. Osteogenic cell populations may be augmented by transplantation of autogenous bone or bone marrow. Homing of osteogenic cells from systemic circulation represents a third possible mechanism. In a prior publication, we found evidence for homing of osteogenic cells; however, the magnitude of homing under settings of normal fracture repair was small (1). This suggests that enhanced homing of osteogenic cells represents a potential therapeutic target. This study was designed to test the hypothesis that signaling molecules known to be active in homing of cultured marrow stromal cells (MSCs) might be used to enhance homing of osteogenic cells into sites of fracture repair, specifically stromal cell-derived Factor-1 (SDF-1) (2) and monocyte chemotactic protein-3 (MCP-3) (3).

Methods
Animal Model: Fifty pairs of transgenic GFP+ and wild type (GFP-) C57BL/6 mice were surgically conjoined (right/left respectively) as parabiotics at 7-8 weeks of age, as previously described (1), in accordance with AAALAC approved procedures. Fibular osteotomy was performed 4 weeks after parabiosis on the left hind limb of the GFP- animal in each pair (Fig 1A). Animals were divided between 5 groups per Table 1.

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<thead>
<tr>
<th>Group</th>
<th>Treatment Approach</th>
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<tr>
<td>1</td>
<td>Fracture</td>
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<tr>
<td>2</td>
<td>Fracture + scaffold</td>
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<tr>
<td>3</td>
<td>Fracture + scaffold seeded with MSCs</td>
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<td>4</td>
<td>Fracture + scaffold seeded with MSCs that express SDF1</td>
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<tr>
<td>5</td>
<td>Fracture + scaffold seeded with MSCs that express MCP3</td>
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Cell Culture and Scaffold Preparation: Delivery of secreted homing molecules was accomplished using syngeneic culture-expanded MSCs isolated from the femur and tibia of 4-5 week old mice. Cells were immunodepleted of CD45 and CD34+ hematopoietic cells using the EasySep PE magnetic cell sorting system (Stem Cell Tech). Stable transfection was accomplished using a replication defective lentivirus encoding either SDF-1 or MCP-3. Cells with stable lentiviral integration were selected in Blasticidin Selection media and expanded at 5% O2 to passage number 9-12. Approximately 6 x 10^5 cells were concentrated in 10 µl of media and pipetted onto a 1 x 1 x 4 mm collagen-based bone substitute, HEALOS II (Depuy Inc, MA). Scaffolds were incubated for three hours to allow cell adherence, brought to the surgical suite under sterile conditions, and implanted adjacent to the fibula, spanning the fracture site.

Histological Assessment: The fracture region and ipsilateral femur were harvested 2 weeks post fracture, the time point previously found to demonstrate maximal prevalence of GFP+ cells (1). Sagittal sections (15 µm) through the fracture callus were stained with VectorRed Alkaline Phosphatase (AP) Substrate solution and Vectashield mounting medium with DAPI and stored at 4°C until imaging. Confocal imaging was performed as described previously (1). Regions of interest were determined by DAPI scans and then subsequent AP and GFP images were obtained. An Image-Pro Plus (Media Cybernetics) macro was designed to determine the number of GFP+/AP+, GFP+/AP-, GFP-/AP+, and GFP-/AP- cells (Fig 1B). AP+ cells within the fracture callus were interpreted to be osteogenic cells (osteoblasts or pre-osteoblasts). GFP+ cells were interpreted to be the cells, or progeny of cells that arrived in the fracture callus from systemic circulation. Double positive cells (GFP+/AP+) were interpreted as osteogenic cells derived from cells recruited through systemic circulation.

Statistical Analysis: A mean value for each variable was determined for each mouse by averaging data from as many images of each callus as could be obtained (between 1 and 5 from each callus). Using SAS’s JMP software, these variables were then compared between treatment groups using one way ANOVA.

Results
An increase in prevalence of GFP+ cells and GFP+/AP+ cells was found both in SDF-1 and MCP-3 treated fractured sites. When compared to fracture calluses treated with non-transfected MSCs, the fracture callus of mice treated with SDF-1 secreting MSCs demonstrated a significant increase in the number of both GFP+ cells (p=0.0003) and GFP+/AP+ cells (p=0.0005). Similarly, calluses treated with MCP-3 secreting MSCs contained significantly more GFP+ (p=0.02) and GFP+/AP+ cells (p=0.01) compared to MSCs alone (Fig 2A & B). Overall, approximately 80% of all GFP+ cells in the fracture sites in all groups were AP+ (Fig 2B) and 15% of all AP+ cells were GFP+ (Fig 2C), with no difference in overall AP+ cell prevalence between groups.

Discussion
Homing pathways with the potential to recruit osteogenic progenitors from systemic circulation to sites of skeletal injury have not been explored. This work does not provide evidence that SDF-1 and/or MCP-3 play a role in homing of osteogenic cells under conditions of normal fracture repair, though this has been suggested in recent studies (4). However, these data do suggest a possible role for one or both of these signaling molecules as therapeutic modulators that may be used to enhance the homing of osteogenic progenitors into sites of bone repair. Ongoing investigation seeks to: characterize the potential role of SDF-1 and MCP-3 in normal fracture repair; refine methods for local delivery of SDF-1 and MCP-3; refine characterization of the GFP+ cell population that is recruited into fracture callus (both osteogenic and non-osteogenic cells).

References