Introduction
Endochondral fracture repair involves the differentiation of multipotential mesenchymal cells into chondrocytes thereby forming the soft callus. While many studies have examined the expression of bone derived growth factors and cytokines at the fracture site (1), few studies have specifically addressed the source of these multipotential mesenchymal cells (2). Three potential sources of cells participating in the repair process are the bone marrow, periosteum, and soft tissue surrounding the fracture site (3). The roles of these individual cell types in the fracture repair process has been largely inferred indirectly through histologic studies localizing expression of RNA and proteins during the repair process (4,5). In this study, we have directly examined the distribution of bone marrow derived cells during the early fracture repair. This was accomplished through the use of cross-sexed bone marrow transplantation and in situ hybridization (FISH) in the rodded fracture in the rodent model. Following fracture, the localization of bone marrow and bone marrow derived cells was determined by identifying the male cells in the female background, and vice-versa.

Materials and Methods
All animal protocols were approved by the animal studies committee at Washington University in accordance with established guidelines. Female C3H/HeJ mice at 7 weeks of age received a lethal dose of gamma irradiation followed by rescue with a tail vein injection of 5 million bone marrow cells in 0.4ml phosphate buffered saline from a male donor. The reciprocal transplant was also performed. Bone marrow cells were harvested by flushing cells from the femora and tibiae of similar age and strain mice. Mice in which the transplanted bone marrow did not engraft died approximately 10 days after irradiation. Ten days following irradiation and transplant, mice were anesthetized and subjected to a prefixed, closed, left tibia fracture following established protocols (6). Non-irradiated, non-transplanted female mice were treated similarly. Mice were euthanized daily between 2 and 7 days following fracture, and the tibia was dissected and processed for paraplast embedding. Male and female cells were identified on the same 5 micron section through fluorescent in situ hybridization (FISH) of sex chromosomes using commercially available pre-labelled probes. Probe for the X chromosome was FITC labeled mouse chromosome X-specific paint (Cambio, 1189-XMF-01), and for the Y chromosome was Cy3 labeled mouse chromosome Y-specific paint (1200-YMCy3). Staining was performed for both chromosomes simultaneously, with DAPI counterstain. Serial sections were also stained with hematoxylin and eosin to evaluate morphology.

Results
Five days following fracture, a cartilagenous callus was present. The cartilage was located a small distance from the fracture site, and observed to be between the bone and surrounding soft tissue. Cells immediately surrounding the fracture site had a spindle-shaped morphology resembling unorganized connective tissue. Most of the cellular reaction was circumferential to the bone. Comparison with control mice, as well as previous studies (6), indicated that the healing process was not affected by irradiation and transplant. By day 7, the cartilage tissue was increased and located more proximally, but not bridging, the fracture site, which still had connective tissue-appearing cells surrounding it. Following FISH staining, female cells could be identified by the presence of two green X chromosome signals, while male cells contained one each red and green. Female mice with male bone marrow showed male cells throughout their bone marrow but not in their chondrocytes and osteocytes distant from the fracture site, as expected. The periosteum away from the fracture was also of female origin. The chondrocytes in the soft callus contained two green signals, suggesting that they were of host, and not bone marrow, origin. However, the connective tissue cells at the fracture site contained cells of both host and donor origin, on both day 5 (figure 1) and 7 following fracture. Similar patterns of host and donor cells were seen in male mice which received female cells.

Discussion
In this study we have directly shown the distribution of bone marrow cells during early fracture repair in the rodded mouse tibia fracture model. On days 5 and 7 following fracture, cells from the bone marrow populated the soft tissue surrounding the fracture site. The chondrocytes forming the soft callus were of host sex suggesting that they did not originate from the bone marrow. The chondrocyte progenitors are thus likely periosteal or surrounding soft tissue cells. The observation of proliferating cells within muscle at the fracture site has been reported (6), and could represent cartilage progenitors. While bone marrow cells appear not to serve as a source of chondrocytes, they do distribute themselves in the soft tissue surrounding the fracture, and may function to secrete cytokines and growth factors to direct the healing process. Identifying genes expressed by these donor cells may allow description of their specific function during early fracture repair. It should be noted that the distribution observed may not apply to all fracture patterns. Understanding the in vivo function of these bone marrow derived cells may lead to improved treatment of fractures as well as other orthopaedic conditions in which bone marrow is being considered for cell-based therapy. In summary, this study represents one of the only investigations in which bone marrow cellular spatiotemporal distribution was directly traced during early fracture repair, and indicates that these cells localize to the soft tissue at the fracture site, but are not the source of multipotential cells undergoing chondrogenesis.

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References