INTRODUCTION:

5 to 10% of the 6 million reported fractures in the United States each year progress to a costly union or a non-union. The overall cost for successful treatment of a non-union of the tibial shaft ranges from $23,246 to $58,525, and return to work occurs at an average of 6 months after non-union operative intervention, in the best-case scenario. Iliac crest autologous graft (ICAG) remains the gold standard to treat non-unions; 200,000 autologous bone grafts are performed annually in the United States. Major complications reported with autologous bone grafts including deep infection, chronic severe pain etc, range from 2.4% to 8.6%, and minor complications (hematoma, chronic mild pain) total approximately 20%. In light of these data, a safe and effective method to treat a non-union without using an ICAG is a current challenge. We and others have previously shown that MSCs are able to migrate from a remote site to the site of injury. However, whether cells from the osteoblast lineage demonstrate systemic recruitment when a fracture occurs is unknown. MC3T3-E1 subclone 14 cells, are a C57BL urine osteoprogenitor cell line with a high level of osteoblast differentiation. We hypothesized that MC3T3-E1 preosteoblast cells can migrate from a remote site to a bone defect to directly enhance bone healing.

METHODS:

Ten, 12 week old nude mice nu/nu were housed and fed in our animal facility. The experimental design was approved by the Institutional Administration Panel for Laboratory Animal Care (APLAC number 9943). We strictly followed university guidelines for care and use of laboratory animals. Animals were divided into two groups. Both groups underwent a unicortical bone defect using a 0.7 mm drill in the middle of the femoral shaft. Group 1 animals (n=5) were injected with 5x10⁶ MC3T3-E1 subclone 14 cells through a left intracardiac injection and Group 2 animals (n=5) were similarly injected with sterile saline solution. The murine MC3T3-E1 subclone 14 pre-osteoblasts were transfected with the lentivirus vector to express the bioluminescent optical reporter gene firefly luciferase (fluc) and a fluorescent reporter gene tomato. Both groups underwent microCT one day before cell injection and at day 14. Bioluminescence (BLI) was performed at day 0, immediately after cell injection and at day 1, 2, 4, 6, 8, 10, and 14. All animals were euthanized after final imaging and their femurs were harvested for immunohistological staining. Bioluminescence data, microCT data and quantitation of positive cells were analyzed by the nonparametric Mann-Whitney U test (two-tailed).

RESULTS:

Successful MC3T3 left intracardiac injection was confirmed with bioluminescence immediately after injection: cell signals were diffusely spread throughout the entire body with slight concentration in the lungs. Reporter MC3T3 cells remotely injected in the left ventricle were systemically recruited toward the bone defect in the left femur. In Group 1, we observed a significant increase of systemic migration of MC3T3 cells at day 6, 8 and 14. Bioluminescence (BLI) was performed at day 0, immediately after cell injection and at day 1, 2, 4, 6, 8, 10, and 14. All animals were euthanized after final imaging and their femurs were harvested for immunohistological staining. Bioluminescence data, microCT data and quantitation of positive cells were analyzed by the nonparametric Mann-Whitney U test (two-tailed).