Local Administration of Non-diabetic hMSCs to Diabetic Murine Femoral Fractures Enhances Callus Remodeling and Deposition of Reparative Bone

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\textbf{Introduction}: Long bone fractures in diabetics are slower to heal, have an increased risk for developing non-union and demonstrate greater potential of infection and perioperative complications compared to non-diabetics. The causative aberrant bone mineral density and insufficient bone microstructure of diabetic patients is thought to result from altered osteoblast and osteocyte function, increased bone marrow adiposity, decreased progenitor osteo- and chondral differentiation potential and increased pro-inflammatory cytokine circulation. It is therefore reasonable to hypothesize the root cause of faulty diabetic bone homeostasis and fracture repair is a reduced population of bone marrow progenitor cells (MSCs) and/or their decreased osteochondral capacity complicated by their repressed neo-vascular potential. The therapeutic efficacy of locally administered non-diabetic human MSCs to support femoral fracture repair in a murine model of diabetes is here investigated.

\textbf{Methods}: All methods involving the use of mice or the harvesting of human bone marrow were ethically and procedurally approved by both the University and Irish Medicines Board. Diabetes was induced in 10 week old male C57/B6 mice by IP administration of 40mg/kg STZ. Three weeks after the onset of diabetes (consistent fasting blood glucose >13mM), a femoral fracture was created by inserting an intramedullary stabilizing pin and then dropping a weight onto the extended leg. Only animals with transverse fractures were admitted to the study. Seven days after fracture creation, 0.5x10\textsuperscript{6} or 1.0x10\textsuperscript{6} human bone marrow derived MSCs (isolated by Orbsen Therapeutics) were administered directly to the fracture. Control mice received an injection of saline. Enrolled mice were monitored weekly for blood glucose by tail vein sampling and fracture callus formation/remodeling by radiography. Fifty six days after fracture, the mice were sacrificed and the femurs assessed by microCT.

\textbf{Results}: The effect of local MSC administration on the diabetic condition was evaluated by weekly monitoring of mouse weight, blood glucose level and terminal levels of circulating HbA1c. No significant change in body weight, blood glucose or HbA1c was observed throughout the 56 day study (Figure 1). Animals maintained their weight at 26-28g, their blood glucose levels >13 mM and HbA1c levels of 10-15% regardless of treatment group.

However, the minimal systemic effect of MSC administration is not due to the clearance of the transplanted MSCs. Although ex-vivo lymphocytic recall assays re-exposing combined inguinal and popliteal lymphocytes to human MSCs does significantly stimulate lymphocytic proliferation(Figure 2), PCR-based analysis of human MSC biodistribution demonstrated the retention of approximately 3% of the administered human cells 2 weeks after administration.

Preliminary microCT analysis indicates an increase on bone volume, a statistically significant increase in bone mineral density and trabecular thickness and a statistically significant decrease in the ratio of bone surface area to bone volume specifically in animals treated with 0.5x10\textsuperscript{6} MSCs as compared to saline...
treated controls, demonstrating an enhancement in fracture repair as a result of cell administration. Interestingly, all indications of fracture repair in mice treated with 1x10^6 cells were regularly repressed as compared to saline-treated controls. The reparative callus reflected a similar trend in that it was smaller in animals treated with 0.5x10^6 cells as compared to saline-treated controls, indicating enhanced remodeling and repair. Ongoing biomechanical analysis using four point bending will further elucidate the potential increase in de novo bone integrity as a result of MSC administration.

**Discussion:** The onset of diabetes in male C57/B6 mice results in the formation of femoral fracture non-union 56 days post-fracture. Although the local administration of human MSCs did not alter the organismal diabetic condition, treatment of the diabetic fracture with specifically 0.5x10^6 MSCs results in enhanced callus resorption, de novo bone formation with an increased mineral density and trabecular structure. Therefore, the local administration of non-diabetic MSCs to diabetic fractures has the potential to enhance callus remodeling resulting in the deposition of higher quality reparative bone.

**Significance:** Current figures estimate 382 million people worldwide have been diagnosed with diabetes, a chronic disorder that comprises the quality of a patient’s skeletal tissue and impedes its ability to repair after fracture. The 56.3 million diabetic patients residing within the European Union therefore result in a significant, unsustainable public health expenditure of €108.1 billion annually. Fragility fractures due to low bone strength have become increasingly recognized as a skeletal complication of diabetes mellitus. As a result of this dysfunctional repair process, long bone fractures in diabetic patients often result in complicated cases of fracture non-union or angulated mal-union, thereby reducing an individual’s mobility and limb function.

![Graph A](image1.png)  ![Graph B](image2.png)  ![Graph C](image3.png)

**Figure 1:** Local administration of MSCs did not alter the diabetic condition. No significant change in A) body weight, B) blood glucose or C) HbA1c was observed throughout the 56 day study. Animals maintained their weight at 26-28g, their blood glucose levels >13 mM and HbA1c levels of 16-15% regardless of treatment group.
Figure 2: Lymphocytic Recall Response. Ex-vivo lymphocytic recall assays re-exposing combined inguinal and popliteal lymphocytes to hMSCs significantly stimulated lymphocytic proliferation.