ABSTRACT INTRODUCTION:

The repair of large bone defects caused by trauma and the treatment of non-unions remain major clinical challenges in the management of orthopaedic trauma patients. In the US alone an estimated 15.3 million fractures receive treatment annually, 5-10% of which are complicated by non-union or delayed union. Delayed or non-union can result in the need for multiple surgical interventions and cause significant patient morbidity with prolonged loss of limb function and in extreme cases amputation. Autologous bone grafting has become the gold standard of treatment for this problem, but this technique produces a limited amount of graft material and carries the additional morbidity of a second incision with the accompanying risks of post-operative pain, neurovascular injury, infection, and other complications. Moreover, clinical studies have demonstrated up to a 30% rate of unsatisfactory results after the operative treatment of trauma-induced segmental bone defects.

As a result, a large body of research has focused on cell-based strategies for bone regeneration as a potential alternative to autologous bone grafting. This field of research has primarily focused on the delivery of mesenchymal stem cells (MSCs) to sites of non-union and critical sized bone defects. Most investigators have used MSCs because of their known ability to differentiate into osteoprogenitor cells, the main cellular mediators of bone formation. However, fracture healing and in particular the healing of bone defects caused by trauma or non-union, requires a coordinated coupling between osteogenesis and angiogenesis, yet the delivery of cellular elements with both potent osteogenic and angiogenic properties remains largely uninvestigated in orthopaedics. Endothelial progenitor cells (EPCs) are a relatively novel cell type of stem cell lineage, which have been shown to participate in postnatal vasculogenesis and respond to tissue ischemia by mobilization from the bone marrow. EPCs have been widely investigated as a mode of ‘therapeutic angiogenesis’ in the fields of cardiovascular disease, peripheral vascular disease, and ischemic stroke, with impressive results in pre-clinical studies leading to clinical trials. Moreover, EPCs have recently been shown to be capable of differentiating into osteogenic cells in vitro and have been shown to be upregulated in response to orthopaedic trauma in humans.

The purpose of this study was to compare the effects of two types of stem/progenitor cells on the healing of critical sized bone defects in a rat model. EPCs, a novel cell type with previously demonstrated effects on angiogenesis in animal models of vascular disease, were compared to both a control group of no cell therapy, and a treatment group of MSCs. The hypothesis was that EPCs would demonstrate both superior bone healing and angiogenesis, when compared to the control group and MSC group.

METHODS:

EPCs and MSCs were isolated from the bone marrow of syngeneic rats by differential culture and grown ex vivo for 10 days. Subsequently the cells were harvested, seeded on a gelmat scaffold, and implanted into a 5mm segmental defect in a rat femur that had been stabilized with a plate and screws. Bone healing was assessed radiographically and by microCT. Angiogenesis was assessed by histology and physiologically, using laser doppler to assess blood flow in the bone and soft tissues. All animal protocols were approved by and performed in accordance with the St. Michael’s Hospital Animal Care Committee. ANOVA was used to test for significant differences between the groups, and a p-value of <0.05 was considered statistically significant.

RESULTS SECTION:

The EPC (n=14) group demonstrated radiographic evidence of healing of the bone defect as early as 2 weeks, and all specimens were radiographically healed at 6 weeks. Both the control group (n=14) and the MSC group (n=14) showed no radiographic evidence of healing at 10 weeks (see Figure 1). MicroCT comparison of the EPC group versus the control group showed significantly greater bone volume and density at the defect site (p<0.001). More blood vessel formation was observed in the EPC group versus the control group on histology at 2 weeks. Laser Doppler assessment showed significantly more soft tissue and bone blood flow at 2 and 3 weeks in the EPC group versus the control group (p=0.021).

DISCUSSION:

The results of this study demonstrate that EPCs are effective as cell-based therapy for healing critical sized bone defects in a rat model. In this model EPCs demonstrated superiority to MSCs with regard to bone healing. In addition, EPCs demonstrated superior angiogenesis over controls in a rat model of fracture healing. These results strongly suggest that EPCs are effective for therapeutic angiogenesis and osteogenesis in fracture healing.

There is a clinical need for effective strategies in the management of traumatic bone defects and non-unions. Investigation into the use of MSCs as an effective alternative to autologous bone grafting has failed to translate into clinical use. It is possible that EPCs are more effective at the regeneration of bone in segmental defects because of their synergistic effect on angiogenesis and osteogenesis. Further research into EPC based therapies for fracture healing is warranted.

REFERENCES: