**INTRODUCTION**

Poor bone quality is a common challenge in orthopaedic reconstructive surgery and typically leads to complications such as non-union in up to 25% of patients. The situation is exacerbated in the elderly who have a decreased capacity for tissue regeneration and repair. Interventions to promote bone regeneration in large skeletal defects using allograft bone are largely ineffective due to its poor osteo-inductive capability. The goal of this study was to determine if bone marrow-derived mesenchymal stem cells (MSC) seeded in dense collagen scaffolds could enhance bone healing in aged rodents.

**METHODS**

_**Ex vivo:**_ MSC were isolated by adhesion to tissue culture plastic from whole bone marrow harvested from the femurs and tibia of healthy young adult donor mice. The cells were expanded in 2D culture _ex vivo_ before seeding at 6 x 10⁵ in hydrated rat tail collagen to form 3D gels. The collagen gels were then subjected to unconfined compression to expel 98% of the fluid thus generating cell-seeded dense collagen scaffolds. The scaffolded gels were monitored on a weekly basis using Alamar Blue® and differentiation was evaluated after 4 weeks using von Kossa/toluidine blue (2).

_**In vivo:**_ All live animal procedures were approved by a McGill University animal use oversight committee. After 5 days of culture MSC-seeded scaffolds, prepared as above, were transplanted into a 3mm x 1mm full thickness defect drilled in the lateral aspect of the RIGHT femur of 12 month old recipient mice. A similar defect in the LEFT femur was either transplanted with an empty scaffold or left with no intervention. Bone repair was evaluated after 4 or 6 weeks using quantitative micro CT imaging and histological analyses. Bone mass and architecture were defined by the parameters bone volume / tissue volume (BV/TV), trabecular pattern factor (Tb.Pf) and trabecular number (Tb.N). After CT imaging un-decalcified samples were embedded at low temperature in MMA, sectioned at 5μm and stained for mineral with von Kossa/toluidine blue (VK/TB), alkaline phosphatase (ALP) and tartrate resistance acid phosphatase (TRAP).

**RESULTS**

_**Ex Vivo:**_ The dense collagen scaffold supported the survival of MSC up to 4 weeks in culture (A). von Kossa staining of mineral deposited by differentiated osteoblasts was seen in the cell-seeded scaffold (B) but not in the empty scaffold (C) after 4 weeks of culture.

_**In Vivo:**_ Quantitative micro CT analysis of bone healing in the femoral defects at 4 weeks post operative showed a trend toward more bone in the RIGHT defect, which received the cell-seeded scaffold, compared with the LEFT defect that received an empty scaffold. The micro CT data (D) was supported by histochemical staining (E) of 5μm sections of plastic embedded bone for mineral (VK/TB), osteoblast (ALP) and osteoclast (TRAP) activity. In addition to more mineral, increased staining for ALP and TRAP in the presence of cell-seeded scaffolds (lower panels) suggested a higher rate of turnover.

<table>
<thead>
<tr>
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<th>LEFT</th>
<th>RIGHT</th>
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<tr>
<td>BV/TV</td>
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**DISCUSSION**

The data provides clear evidence that dense collagen scaffolds can support the growth and differentiation of MSC _ex vivo_. When transplanted in vivo the cell-seeded scaffolds enhanced bone regeneration in a rodent model of fracture repair. The presence of TRAP activity within the defect occupied by the MSC-seeded scaffold indicates infiltration by catabolic cells, which was not evident in the case of the empty scaffold. MSC-seeded dense collagen scaffolds thus show promise as a potential substitute for bone grafts for bone reconstruction.

**REFERENCES**


**ACKNOWLEDGEMENTS**