Endothelial Progenitor Cells Promote Fracture Healing in a Segmental Bone Defect

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INTRODUCTION:
Vascular ingrowth at the fracture site has a cardinal role in the healing process and regeneration of the bone post fracture.1 Segmental bone defects after severe trauma, infection, and surgical removal of tumors remains a major clinical problem to be addressed. Endothelial Progenitor Cells (EPCs) have the ability to differentiate in vitro into endothelial cells and to contribute to formation of new blood vessels.2 The objective of this study was to evaluate the effects of local use of ex vivo expanded endothelial progenitor cells (EPCs) on fracture healing in a defect model in the rat femur.

METHODS:
Male Fisher-344 rats (250-300 gr.) were used as the animal model for this study. All handling and treatment procedures were approved by St. Michael’s Hospital Institutional Animal Care and Use Committee. Rat bone marrow EPCs were isolated and cultured for 7 to 10 days in endothelial cell growth medium (EGM2-MV). A segmental bone defect (5 mm.) was created in the rat femur diaphysis and fixed with a mini-plate. A gelfoam piece impregnated with a solution of rat bone marrow EPCs (1x106) was placed into the fracture gap. Control animals received only saline-gelfoam with no cells. In total, 14 rats were studied: 7 in EPC and 7 in control group. Animals were sacrificed at 10 weeks post-operatively. Healing of the defect was evaluated with postero-anterior plain radiographs, quantitative micro computed tomography (micro-CT) scans, and histological specimens prepared from the osteotomy site.

Radiological Assessment: Evaluation of the radiographs of the operated femurs was performed according to the presence or absence of bridging callus formation between both ends of the osteotomy gap with complete bone filling of the defect.

Micro-CT Assessment: After scanning of the femur samples, a region of interest including the osteotomy site was determined and quantification of bone morphometry was performed.

Histological Assessment: Paraﬃn blocks of the samples were cut into 5-μm sections and prepared slides were stained with hematoxylin and eosin (H&E). Evaluation of the slides was done qualitatively at 20× magniﬁcation for comparison of the EPC group with the controls according to the amount of new bone formation.

RESULTS:
Radiographically; all 7 animals in the EPC-treated group had complete union with dense callus filling the entire osteotomy gap, whereas in the control group none of the animals had union or bridging callus formation (Fig 1).

Micro-CT assessment showed signiﬁcantly improved parameters of bone volume (36.58 to 10.57 mm3, p=0.000), bone volume density (0.26 to 0.17 %, p=0.000), model index (-2.22 to 2.79, p=0.000), trabecular number (1.28 to 0.91 /mm, p=0.063), trabecular thickness (0.21 to 0.15 mm, p=0.001), trabecular spacing (0.63 to 1.07 mm, p=0.022), bone surface (353.75 to 152.08 mm, p=0.000), and bone surface to bone volume ratio (9.54 to 14.24 /mm, p=0.004) for the EPC group compared to control respectively (Fig 2).

CONCLUSION:
In conclusion, local use of ex vivo expanded endothelial progenitor cells signiﬁcantly enhanced bone healing in a segmental defect model in rat femur diaphysis compared with that in control animals. On the basis of these results, local use of EPCs may be introduced as a promising cell-based therapy to promote bone regeneration at a fracture site.

REFERENCES:

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Figure 1. X-rays of the operated femurs from the EPC (upper row) and the control group (lower row) at 10 weeks. (Arrows indicating the osteotomy site)

Figure 2. Micro-CT images showing complete union and filling of the defect with new bone in the EPC group (upper row), compared to insufficient bone formation in the control group (lower row).

Figure 3. Histology sections of the osteotomy gap and the adjacent bone at 10 weeks (arrows indicating osteotomy edges) from the EPC (left) and the control group (right). (H&E; x2)