INTRODUCTION: Severe fracture damages vessels and disrupts circulation at the fracture site that leads to acute necrosis and hypoxia of adjacent bone and marrow contents. This leads to an increased risk of poor fracture healing. We hypothesized that 1) Cell-based VEGF gene transfer without viral vectors locally may function as a factory to produce VEGF protein at a fracture site. 2) VEGF may couple angiogenesis and osteogenesis to accelerate fracture healing. The objective of this study was to develop a cell-based VEGF gene therapy in order to accelerate fracture healing and investigate the effect of VEGF in bone repair with a small animal model.

METHODS: Animal model: All animal procedures were approved and performed in accordance with the Animal Care Committee at St. Michael’s hospital. 21 New Zealand White male rabbits were studied. After anesthesia, the right tibia was exposed through an anterolateral approach and, through extraperiosteal dissection, circumferentially freed from the surrounding muscles. A 10 millimeter segmental bone defect was created after 12 mm periosteal excision in the middle 1/3 of each tibia. The fracture was stabilized with a stainless steel plate (2.7 mm 9 hole DCP). Experimental groups by local injection were: 1) Transfected fibroblasts with VEGF (n=7), 2) Fibroblasts alone (n=7), and 3) PBS only (n=7). Radiographs were taken at every 2 weeks for assessment of the process of fracture healing. The animals were sacrificed and the fracture healing specimens were collected at 10 weeks post surgery for histology, immunohistochemistry and micro-CT scan analysis. After removal of the plate and surrounding soft tissue, the fracture healing specimens were fixed in periodate-lysine-parafomaldehyde (PLP) fixative, decalcified in an EDTA-G solution, and embedded in paraffin. Sagittal ground sections were prepared (5 um) and stained with hematoxylin/eosin (HE) or CD31. Before decalcification, the specimens were scanned with Micro-CT. Cell-based gene transfer of VEGF [1, 2]: The full length coding sequence of human VEGF was generated. Primary cultured rabbit fibroblasts, from the same rabbit as the one which was injected with the autogenic cells, were transfected by use of SuperFect (Qiagen Inc) with VEGF-pcDNA 3.1. The transfected cells with VEGF were collected at 24 hours after transfection. 5.0 X 10^6 cells in 1 ml PBS were delivered via impregnated gelfoam into the fracture site and injected into the surrounding tissues.

RESULTS: Radiology: Fracture healing was defined as those with bone bridging of the fracture defect. After 10 weeks, fourteen tibial fractures were healed in total including six in group 1, four in group 2 and four in group 3. The VEGF group had an earlier initial sufficient volume of bridging new bone formation which was confirmed by the histological assessment and micro-CT imaging. Histological evaluation demonstrated the ossification across the entire defect in response to the VEGF gene therapy, whereas the defects were predominantly fibrotic and sparsely ossified in groups 2 and 3 (Figure 2). Micro-CT evaluation of the new bone structural parameters showed that the amount of new bone (volume of bone (VolB) x bone mineral density (BMD)), bone volume fractions (BVF), bone volume/tissues (BV/TV), trabecular thickness (Tb.Th), number (Tb.N) and connectivity density (Euler number) were higher; while structure model index (SMI), bone surface/bone volume (BS/BV), and trabecular separations (Tb.Sp) were lower in the VEGF group than the other groups. P-Values <0.05 indicated statistical significance (ANOVA, SPSS) in all parameters except for SMI (0.089) and VolBxBMD (0.197). Numerous positively stained (CD31) vessels were shown in the VEGF group (Figure 4)

DISCUSSION: We sought to determine whether local delivery of the VEGF gene by cell based transfer could be done to promote fracture healing. These results indicate that VEGF gene delivery by the cells have significant osteogenic and angiogenic effects in this model. These data encourage the further development of genetic approaches to the augmentation of bone healing. The experiments presented here demonstrate the ability of cell based VEGF gene therapy to enhance healing of a critical sized defect in a long bone in rabbits.


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