The Effect of Cryopreserved Endothelial Progenitor Cells on Bone Healing in a Critical Size Bone Defect Animal Model: A Radiographic, Micro-Computed Tomography and Biomechanical Analysis

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INTRODUCTION: Endothelial progenitor cells (EPCs) are a highly effective cell-based therapy for fracture healing. However, their use is limited by the need for appropriately timed ex-vivo cell isolation and expansion. Cryopreservation represents a promising strategy to overcome this limitation by enabling the long-term storage of EPCs. Thus, the purpose of this study was to compare the therapeutic potential of EPCs before and after cryopreservation in a rat model of a critical size bone defect.

METHODS: EPCs were isolated from the bone marrow of donor Fischer 344 rats and cultured for 8 days. On day 8, half of the cells (fresh EPCs) were separated into groups of $2x10^6$ and loaded on a gelatin scaffold. The remainder of the cells (cryopreserved EPCs) were cryopreserved for 7 days prior to thawing and loading on an identical gelatin scaffold. To compare the effectiveness of fresh compared with cryopreserved EPCs on bone healing, 5 millimeter segmental defects were created in the right femora of Fischer 344 rats, followed by stabilization with a miniplate and screws. Rats received one of the following treatments: i) $2x10^6$ fresh EPCs (n = 7) or ii) $2x10^6$ cryopreserved EPCs (n = 9) delivered on a gelatin scaffold. Bone healing progression was monitored through biweekly radiographs of the operated femora. Radiographs were scored and assessed for union status and scored for degree of bone healing by two orthopaedic surgeons blinded to treatment group. All animals were euthanized 10 weeks after the surgery. The operated femora were then evaluated using micro-computed tomography (micro-CT) and biomechanical testing.

RESULTS: All animals treated with fresh (n=7/7) and cryopreserved (n=9/9) EPCs achieved full union at 10 weeks. Animals treated with fresh EPCs had significantly higher radiographic scores at 2 weeks ($p < 0.05$), but showed no statistically significant differences thereafter ($p > 0.05$). Micro-CT analysis of the operated femora showed no statistically significant differences between groups for bone volume (BV) or bone volume normalized to total volume (BV/TV; $p > 0.05$), with excellent bone formation in both groups. Finally, biomechanical testing of the femora showed no differences in maximum stiffness, maximum torque, and yield point between the treatment groups ($p > 0.05$).

DISCUSSION AND CONCLUSION: These results demonstrate that cryopreserved EPCs are highly effective and equivalent to fresh EPCs for healing critical size bone defects in a rat model of nonunion. These results support the concept that cryopreservation may allow for “off-the-shelf” availability of EPC-based therapies.