A Radiographic and Micro-CT Analysis of the Effects of Cryopreserved Endothelial Progenitor Cells on Bone Healing in a Critical Size Bone Defect Animal Model

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Purpose: 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 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. Based on in vitro evidence demonstrating preserved EPC characteristics and functionality following cryopreservation, we hypothesized that cryopreservation would not impair EPC fracture healing capability.

Methods: 5-mm segmental defects were created and stabilized with a mini plate and screws in the femora of male Fischer 344 rats. Subsequently, animals received fresh EPCs (n = 7) or cryopreserved EPCs (n = 9). The 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 delivered into the femoral defects on a gel foam sponge (2 x 10^6 each) and half of the cells were cryopreserved for 7 days at –80°C prior to being thawed and then inserted into identical femoral defects. Bone formation was assessed with biweekly radiographs and micro-computed tomography (micro-CT) following euthanasia at 10 weeks after the surgery.

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 (see Figure 1).

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. This supports the concept that cryopreservation may allow for "off-the-shelf" availability of EPC-based therapies. Further research into the use of cryopreserved EPCs is warranted.