

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

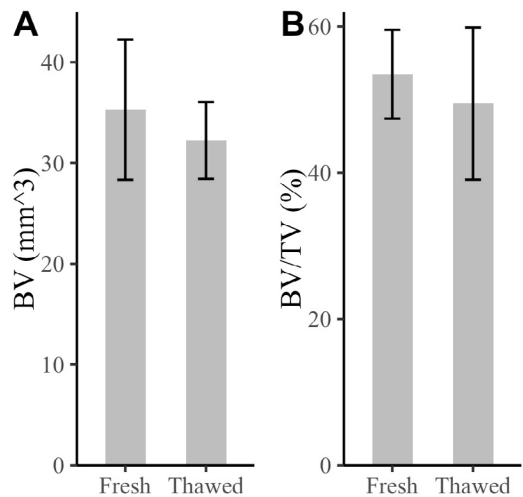
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Purpose: Endothelial progenitor cells (EPCs) are a highly effective cell-based therapy for fracture healing. However, their use is limited by the need for appropriately timed cell isolation and expansion. Cryopreservation represents a promising strategy 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 (2×10^6 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.



The FDA has stated that it is the responsibility of the physician to determine the FDA clearance status of each drug or medical device they wish to use in clinical practice.