The effect of cryopreserved endothelial progenitor cells on bone healing in a critical size bone defect animal model

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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: All animal procedures were approved by the Institutional Animal Care Committee at St. Michael's Hospital and were conducted according to their guidelines. 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 versus 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 assessed for union status and scored for the amount 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 (Figure 1). 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 ($p > 0.05$, Figure 2).

DISCUSSION: The results of this study demonstrate that there are no differences in radiographic, micro-CT or biomechanical outcomes of bone healing when comparing animals treated with fresh versus cryopreserved EPCs. Thus, cryopreservation does not appear to impact the bone healing capacity of EPCs and cryopreserved cells are highly effective at healing critical sized bone defects.

CLINICAL RELEVANCE: This study demonstrates that cryopreservation of EPCs for bone healing therapy is a viable and highly effective strategy. This finding will greatly facilitate the clinical translation of EPC therapy for the treatment of non-healing fractures.

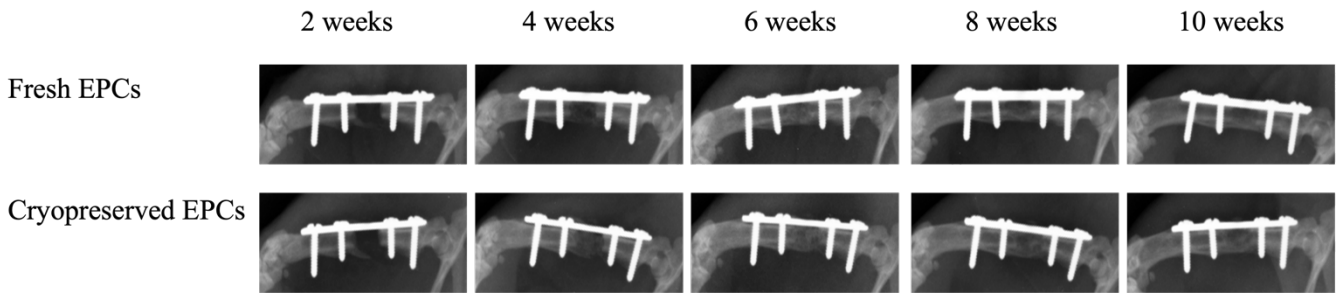

Figure 1: Representative anteroposterior radiographic images following Fresh and Cryopreserved EPC implantation into the defect site.

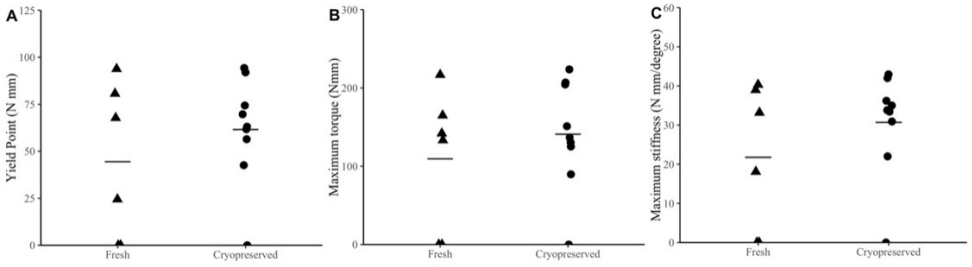

Figure 2: Biomechanical properties of the operated femora in animals receiving Fresh and Cryopreserved EPCs: (A) yield point, (B) maximum torque, and (C) maximum stiffness. Each symbol represents an individual outcome. The horizontal lines represent the mean for each group. There were no significant differences between the two groups.