Δ Investigating an Endothelial Progenitor Cell Dose Response for the Healing of Critical Size Bone Defects

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Background/Purpose: The management of bone defects and nonhealing fractures remains a considerable challenge for orthopaedic surgeons. To date, the preferred method of treatment still remains the autologous iliac crest bone graft (AICBG). Unfortunately, AICBG is associated with excess patient morbidity, a risk of infection, and a suboptimal rate of success. Researchers have investigated the use of bone cell precursors as well as growth factor therapies in an effort to remedy this situation, but these approaches have had limited success clinically. Within recent years, our group on the other hand has shown consistent success in repairing critical size defects in the femurs of rats using a bone marrow-derived endothelial progenitor cell (EPC). EPCs have been shown to promote and enhance both osteogenesis and angiogenesis—critical components of fracture healing. The purpose of this study is to therefore further explore and optimize this cell therapy by investigating an EPC dose-response relationship for bone healing in a rat model. We hypothesize that the local application of EPCs to a nonhealing defect will improve bone healing in a dose-dependent manner until a plateau of effectiveness is reached.

Methods: Male inbred rats underwent a double osteotomy of their right femur to create a 5-mm nonhealing defect, which was subsequently stabilized by a mini-plate and screws. A biodegradable collagen scaffold seeded with varying doses of syngeneic, ex vivo expanded EPCs (100,000, 500,000, 1 million, 2 million, or 4 million cells; n = 6), was then placed into the defect before the wound was carefully sutured. The cells used for implantation were isolated from a separate sacrificed rat whose bone marrow was cultured in endothelial growth media for approximately 7 days prior to surgery. To monitor the progress of bone healing, biweekly radiographs of the operated femur were taken up until our 10-week end point and sacrifice. These radiographs were then scored by blinded assessors according to the proportion of the defect filled with callus and its density. Postsacrifice, micro-CT analysis and biomechanical testing were then used to further evaluate and quantify bone healing. All animal protocols were approved by the St. Michael's Hospital Animal Care Committee.

Results: As evidenced by our scored radiographs, earlier bone healing and union was observed in animals that received our largest dose of EPCs, 4 million. The average time to union in this high-dose treatment group was 4 weeks—2 weeks faster than the next quickest groups (1 and 2 million EPCs). Yet, micro-CT analysis and biomechanical testing revealed that animals that received 2 million EPCs experienced the greatest amount of bone formation, and the greatest biomechanical strength (Fig. 1). Crucially, the femurs of animals that were treated with 2 million EPCs also showcased strengths that were not significantly different than intact, nonoperated femurs ($P < 0.05$).

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See pages 49 - 106 for financial disclosure information.

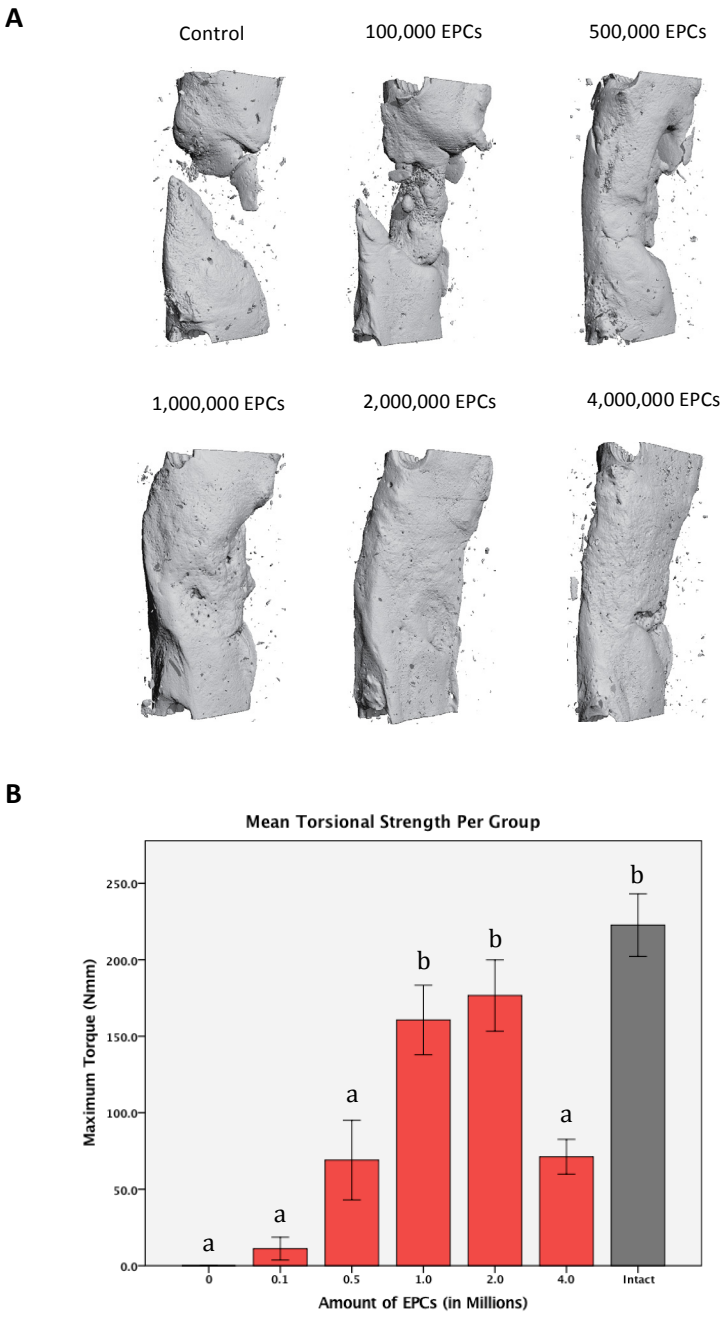

Figure 1. (A) MicroCT 3D reconstructions of the defect area showing differences in bone healing across the various treatment groups. **(B)** Graphical representation of the maximum torque sustained by specimen in each treatment group, including intact femurs. Error bars represent ± SE; different letters denote significance ($p<0.05$).

Conclusion: To date, the optimal concentration of bone marrow-derived EPCs for bone healing has yet to be elucidated. From our results, we conclude that earlier, but not functionally superior, bone healing occurs when 4 million EPCs are applied to a 5-mm bone defect. The greater biomechanical strength and bone volume observed in animals receiving a submaximal dose of cells in this investigation (2 million EPCs) suggests a peak of effectiveness for EPC therapy, contrary to our initial hypothesis. Overall, the results of this study highlight the importance of appropriate cell dosing in tissue repair, while also guiding future investigations in their design of bone regenerating EPC-based therapies.

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