Biological Augmentation of Rotator Cuff Repair with Endothelial Progenitor Cells

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Introduction: Acute and chronic rotator cuff tears represent a substantial clinical burden in our society and remain an unsolved problem in orthopaedics. Despite the evolution of minimally-invasive surgical techniques for rotator cuff repair, including mini-open and arthroscopic approaches, failure of surgically repaired rotator cuff tears to heal continues to represent a significant problem. Estimates of postoperative re-tear rates range from 11-94%,¹ and failure of rotator cuff healing is correlated with inferior clinical outcomes. This has lead to significant research interest in biological strategies for the augmentation of surgical repair, with numerous pre-clinical and clinical studies evaluating a variety of strategies including growth factor supplementation, application of progenitor cells, and the use of extracellular matrices. Despite this, an effective biological strategy for the augmentation of rotator cuff repair remains elusive. Among the strategies being currently investigated, the use of autologous cell-based strategies is of particular interest. However, recent pre-clinical investigation of cell-based strategies employing mesenchymal stem cells (MSCs) has produced disappointing results.²

Hypovascularity of the native tendon is felt to be a contributing factor to the etiology of rotator cuff tears and impaired vascularity at the desired site of tendon-bone healing may be the most significant element in failed rotator cuff healing.³ It is possible that failure to address this vascularity is the reason why previous biologic strategies have failed. Endothelial progenitor cells (EPCs) are a relatively novel cell type of stem cell lineage, which have been shown to participate in postnatal vasculogenesis and respond to tissue ischemia by mobilization from the bone marrow.⁴ EPCs have been widely investigated as a mode of ‘therapeutic angiogenesis’ in the fields of cardiovascular disease, peripheral vascular disease, and ischemic stroke, with impressive results in pre-clinical studies leading to clinical trials.⁵ Recent investigation of EPCs in fracture healing has demonstrated that EPC therapy significantly enhances fracture healing and angiogenesis in numerous animal models.⁶ ⁷ Given the documented capacity of EPC therapy to stimulate both fracture healing and angiogenesis, it stands to reason that the application of autologous EPCs to a desired site of tendon-bone healing, such as a rotator cuff repair model, may improve the healing response. However, EPC therapy for the augmentation of rotator cuff repair has never been investigated. The current study sought to address this by investigating the use of autologous EPCs in a rotator cuff repair model. We hypothesized that the therapeutic application of EPCs would increase local vascularity at the tendon-bone interface, and thereby improve rotator cuff healing.

Methods: A syngeneic population of Fischer 344 rats was used in this study to allow autologous transplantation of cells. All procedures were approved by the Animal Care Committee at St. Michael’s Hospital. EPCs were isolated from the bone marrow via Ficoll density separation, fibronectin adherence, and endothelial growth medium (EGM2-MV) supplementation, as previously described.⁶ The cell
population was cultured and expanded ex-vivo for 11 days prior to transplantation. These cells were verified to be an appropriate EPC population using flow cytometry with antibodies against CD34, CD133, and Flk-1 surface antigens. In all animals, the rotator cuff (supraspinatus) was detached and subsequently repaired using transosseous sutures. Animals in the EPC group received one million cells suspended in fibrin sealant and placed at the bone tendon interface prior to final repair. Control animals received fibrin sealant only at the bone tendon interface. Animals were sacrificed at 2, 4, and 6 weeks. The primary outcome measure of the study was the functional strength of the tendon-bone attachment as assessed with biomechanical testing. Biomechanical testing was performed using previously described methods in the rat rotator cuff repair model. Briefly, a uniaxial testing system with a customized jig was used to test the pull-out strength of the supraspinatous tendon repair. Maximum load-to-failure and stiffness was recorded for each specimen and compared between treatment groups. Supplementary outcome measures included histomorphometric analysis of the organization of collagenous tissue and immunohistochemistry analysis of new blood vessel formation. In addition, green fluorescent protein (GFP)-labelled EPCs were used for cell survival studies after surgical transplantation.

**Results:** Flow cytometry indicated 95% of the cell population was positive for the three surface antigens: CD34, CD133, and Flk-1. The population was an EPC-enriched population. We found that there was no significant difference between the Fibrin group and the EPC group in terms of their tendon attachment strength, stiffness, insertion area, and ultimate stress at two and four weeks. However, at six weeks, there was a significant increase in biomechanical strength at the tendon healing site in favour of the EPC group (see figure 1). There were no significant differences in collagen organization at any time point, however blood vessel density at the tendon repair site at six weeks was increased in the EPC group, although the difference was not statistically significant (see figure 2). Histological analyses demonstrate cell viability at both two and four weeks after surgical transplantation (see figure 3).

**Discussion:** Our results showed a significant increase in healing strength at 6 weeks when autologous EPCs were applied to a rotator cuff repair model and strong trend toward increased vascularity at the healing site. Enhanced vascularity at the repair site may represent the mechanism by which EPCs result in superior attachment strength in rotator cuff healing. These results suggest that there exists significant potential for the therapeutic augmentation of rotator cuff healing with EPCs and further investigation is warranted.

**Significance:** We found that the transplantation of autologous endothelial progenitor cells during rotator cuff repair resulted in improved biomechanical strength and enhanced vascularity at the healing site. These results suggest substantial promise for the use of EPCs for the biological augmentation of rotator cuff repair.
Figure 1. Graphical representation of biomechanical results indicated no differences at two and four weeks, but statistically significant increases in both ultimate load and stress in the EPC treated group six weeks post surgery.

Figure 2. Left: Graphical representation demonstrating no difference in collagen organization at any time point. Right: Graphical representation demonstrating a strong trend towards increased vascular density in the EPC group at 6 weeks on the basis of immunohistochemistry (the difference was not statistically significant).
Figure 3 – 10X magnification indicating GFP-positive cells (indicated by the arrows) in the endothelium 2 and 4 weeks post surgery

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