Local Versus Systemic Delivery of Endothelial Progenitor Cells for a Tissue Scaffold

J.W. McCullars, S.T. Moore, J.A. Jennings, and D. S. Feldman
Department of Biomedical Engineering, University of Alabama at Birmingham, Birmingham, AL 35294

Introduction

Revascularization of chronic wounds, such as pressure ulcers, or even tissue scaffolds is essential for protein production, collagen synthesis, and the destruction of bacteria. Poor blood supply is a rate-limiting step in the healing process. Attempts have been made to improve revascularization through angiogenesis—the formation of new blood vessels from the pre-existing network of vessels. However, angiogenesis is limited in that mature endothelial cells are already fully differentiated, have limited life span and proliferative ability, and have a reduced ability to incorporate into remote sites of ischemia.

Recent studies have shown that vasculogenesis is also an important mechanism for revascularization of adult tissues. Vasculogenesis is defined as the formation of vessels from bone marrow-derived endothelial progenitor cells, or EPCs. EPCs, isolated from bone marrow, peripheral blood, or umbilical cord blood, have the ability to home to sites of tissue damage in the body, differentiate into mature endothelial cells, and incorporate into new vessel structures..

The goal of this study was to compare the effectiveness of using these EPCs to stimulate healing when seeded locally in a tissue scaffold versus injected systemically. . Specifically, it was hypothesized that using the EPCs locally would speed the healing process by stimulating both angiogenesis and vasculogenesis. Additionally, it was hypothesized that combining the local application of EPCs seeded into an albumin scaffold with a systemic injection of EPCs would be the best method for improving healing, by both increasing the number of EPCs in the blood traveling to the wound site and by reducing the amount of time required for the EPCs to initiate vasculogenesis at the wound site.

Materials and Methods

Peripheral blood mononuclear cells were isolated from rabbit and human blood by density gradient centrifugation with Histopaque 1077 (Sigma, St. Louis, MO). Human endothelial progenitor cells were further isolated by magnetic cell separation with the MACS direct CD34 isolation kit (Miltenyi Biotec, Auburn, CA).

PEG-crosslinked albumin scaffolds were made by mixing equal amounts of protein solution and PEG solution. The protein solution contained lyophilized fraction V albumin (Sigma), at a concentration of 0.33 g/ml, reconstituted in 0.85% NaCl solution. The PEG solution was formed by dissolving 0.1 g/ml poly(ethylene glycol) disuccinimidyl glutarate, molecular weight 10K (SunBio PEG-SHOP, Anyang City, S. Korea), in HEPES buffer solution (pH 9.2).

For EPC-seeded scaffolds, isolated EPCs were mixed into the albumin protein solution. Then the albumin and PEG were mixed to form a crosslinked gel containing EPCs evenly distributed throughout.

Four 4 cm x 4 cm full-thickness wounds extending down to, but not through, the panniculus carnosum muscle were created on the dorsum. Each animal had four different wounds: control (C), albumin scaffold (A), albumin scaffold + autologous rabbit PBMC (AR), or albumin scaffold + human $CD34^+$ EPCs (AH). Additionally, half of the animals received a systemic injection of autologous rabbit PBMC (approximately 2 x 10^7 cells) through the ear vein immediately following surgery. These wounds were labeled I, AI, ARI, and AHI to correspond to the non-injected animals. There were five animals of each type per time period.

The wounds were covered with an occlusive dressing (Tegaderm, Cardinal Health, Dublin, OH). Animals were euthanized with an overdose of pentobarbital at the appropriate time (2 or 3 weeks with 5 animals in each group). The wounds were removed and fixed in 10% neutral buffered formalin.

Image analysis software was used to calculate healing rates at each time period using the following equation:

$$\text{Healing rate} = \Delta SA / (P_{avg} \times \Delta t)$$

where SA is wound area and P_{avg} is the average perimeter over the time period (Δt).

Epithelialization rate (ER), the lengths of the new epithelial layer on both sides of the wound and contraction rate (CR) the change in wound diameter over time (between the vertical lines) were calculated similarly from histological slides (Figure 1). A stereological point counting method was used to determine the volume fractions of blood vessels and cell nuclei (fibroblasts, macrophages, and neutrophils).

Results and Discussion

As hypothesized, the use of EPCs both locally and systemically were able to improve the healing rate, in some cases. Interestingly, the injections had the most influence at the 2-week period, while the local EPC scaffolds alone were best during the 3-week period.

Specifically, at 2 weeks the injection alone was best for increasing ER and HR, while the combination of local and systemic treatment was best at decreasing CR and the CR/ER ratio. While the injection alone increased ER, it also increased CR. Overall, ARI was the best treatment at 2 weeks, increasing ER while decreasing CR and CR/ER.

The hypothesis that local EPC treatment alone would improve healing was also proved, particularly for the 3-week time period. At 3 weeks, AR was the best overall treatment, increasing ER, HR,, but with an increase in CR. While local application of rabbit EPCs was successful at improving healing, local application of human EPCs was not.

Future studies will examine other strategies to increase the number of EPCs that actively participate in the local healing response of tissue scaffolds. This will include strategies to increase the systemic levels of EPCs, using multiple injections of EPCs both systemically and locally. In addition, ongoing studies are examining methods to get stem cells to overproduce factors that would help in the healing response.

Acknowledgements

This work was supported by a CDC grant and an NSF Graduate Research Fellowship.

Figure 1. Histological section of a healing wound