Derivation of Human Microvascular Endothelial Cells for Prosthetic Vascular Graft Seeding

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This report summarizes our techniques and experiences deriving microvascular endothelial cells from fat for vascular graft seeding in 17 patients. Microvascular endothelial cells were derived from abdominal wall fat by collagenase incubation. The mean number of cells obtained using the described procedure was \(6.83 \times 10^5\) cells/gram of fat processed. Histologic evaluation of the harvested cells revealed significant numbers of contaminating cell types in addition to Factor VIII-positive microvascular endothelial cells. These cells were seeded onto 6 mm ID PTFE vascular grafts in patients undergoing peripheral vascular arterial revascularization. The mean number of seeded cells was \(8.04 \times 10^6\) cells/graft. Approximately 90 minutes were required to harvest and isolate the microvascular cells from the fat samples. We feel there are significant technical advantages to deriving endothelial cells from microvessels of human fat for vascular graft seeding.

KEY WORDS: Endothelial seeding; microvascular endothelial cells; grafts.

The data that have accumulated from animal studies convincingly argue that endothelial cell seeding (ECS) enhances small-diameter vascular graft performance [1–5]. Based upon the success of the animal studies, the idea of extending the application of ECS to human vascular grafting is attractive. However, among many surgeons there remains an unwillingness to transfer the technology of ECS to human vascular grafting. One reason for this reluctance is that the functional changes occurring in endothelial cells (EC) consequent to graft seeding remain largely unknown. The suggestion has been made that ECS might phenotypically modulate EC from an actively nonthrombogenic phenotype into one which is actively prothrombogenic [6]. In addition, some key technical issues in applying the methods of experimental ECS to the human clinical situation lay unresolved. For example, the most appropriate material surface upon which to seed EC, as well as the optimal method of seeding to promote cell attachment and retention are still unknown. The effects of pore sizes upon endothelial cell seeding and vascular graft healing are only now being vigorously investigated. Furthermore, once implanted, there is little consensus on how to most effectively follow the maturation and progression of endothelial cell seeded prostheses in humans.

The selection of native vessels from which EC might be harvested for human graft seeding also remains an unresolved issue. It could be intuitively argued that EC derived from arteries represent the most biologically appropriate cells for seeding prosthetic arterial conduits. However, in human patients there are no nonessential arteries available for cell derivation. In addition, only a limited number of large veins are available clinically from which...
cells can be derived. Recent reports have demonstrated the feasibility of creating preformed confluent EC monolayers on vascular grafts in short time periods using in vitro perfusion circuits [5,7]. However, in vitro environments may induce phenotypic and even genotypic changes in EC which may negate the theoretical advantages of confluency at the time of graft implantation.

As an alternative to arterial or venous-derived EC for graft seeding, Jarrell and associates [8] suggested the derivation of endothelial cells from the microvessels of fat for graft seeding. The high cell yield that can be obtained from fat processing, in addition to the adherence characteristics of these cells upon prosthetic grafts, offers the possibility of rapid and complete endothelialization of synthetic prostheses in short postoperative times without in vitro perfusion. Pearce and associates [9] first reported successful seeding of 60μm pore size PTFE grafts with microvascular endothelial cells (MVEC) derived from omentum. In their study seven of the 12 PTFE grafts seeded with MVEC were patent. We recently reported our results using MVEC for seeding 4 mm ID Dacron vascular grafts evaluated in canine carotid arteries [10]. Sterpetti and colleagues [11] compared the performances of high porosity PTFE grafts seeded with vein-derived EC and omental MVEC.

The purpose of this paper is to describe the technique that we have recently utilized to derive EC from fat samples in patients undergoing vascular grafting procedures.

MATERIALS AND METHODS

Since January, 1987, 17 patients have undergone peripheral vascular reconstructions at Akron City Hospital in which 6 mm ID Gore-Tex PTFE grafts were seeded with MVEC obtained from samples of abdominal fat. The operations were all leg-saving procedures in patients that did not have available autologous veins to use as bypass grafts. The patients all agreed to the procedure by informed consent. In each patient the initial procedure was to remove a large mass of anterior wall abdominal fat through a small incision. The mass of fat was divided to fit into sterile 50 ml polypropylene centrifuge tubes containing phosphate buffered saline (PBS) for transport to a sterile laminar flow hood for processing. The technique for the isolation of fat-derived microvascular endothelial cells was a modification of the procedures published by Williams and co-workers [12].

The fat samples were transferred from their transport tubes into sterile glass Petri dishes for initial dissections. Fat which was as free of grossly visible blood vessels and connective tissue as possible was dissected free from the mass of fat and transferred to a second Petri dish for mincing. In the first four patients less than 7 grams of fat were available for subsequent processing. In the following six patients 10 grams of "clean" fat were available and in the final seven patients in this series at least 15 grams of fat were dissected out.

The goal has been to process as large a sample as possible in order to obtain the greatest number of endothelial cells for seeding in each patient. The "clean" fat samples were minced using two scalps until the fat was of "cream of wheat" consistency. Approximately 15 minutes of mincing were required. The minced fat was then transferred into a small Erlenmeyer flask and 2 ml of collagenase (Sigma Type IA, Lot #76F-6825) were added per gram of fat. The working collagenase solution was prepared prior to the receipt of the fat sample by dissolving 50 mg of 600 U/mg sterile collagenase in 20 cc of phosphate buffered saline containing calcium and magnesium. The collagenase solution was warmed to 37°C prior to use. The collagenase/fat mixture was then incubated at 37°C in a shaker water bath at 100 strokes/minute for 20 minutes. At the conclusion of the incubation period the collagenase/fat suspension was pipetted repeatedly and transferred into centrifuge tubes which were centrifuged at 100 x g for 10 minutes.

Following centrifugation three distinct layers were visible in each centrifuge tube: the top layer consisted of fat, the middle layer was the collagenase solution and the pellet of microvascular cells was layered in the bottom of the tube. From each tube the fat was aspirated into a pipet and the collagenase was decanted into a waste container leaving the pellet of microvascular cells. These cells were resuspended in PBS, the suspension of cells was pipetted repeatedly and any large masses of connective tissues were removed. The cell suspension was then recentrifuged at 100 x g for 6 minutes and the PBS decanted. The cells were then resuspended in 20 ml of the patient's plasma. Aliquots of the cells were reserved for counting and for histologic processing to analyze the relative proportion of endothelial cells to contaminating cell types. The remaining cells were returned to the operating room for seeding onto the graft.

RESULTS AND DISCUSSION

Table I illustrates the numbers of microvascular cells obtained per gram of fat processed as well as the total numbers of cells seeded on the grafts for each of the 17 patients in this series. The mean number of cells obtained per gram of fat was 6.83 x 10^5 with the range of counts between 3.07-11.56 x 10^5. The variability in cell counts was clearly not a reflection of differences in collagenase activity as the same lot and concentration of collagenase were