Delivery Site of Perivascular Endothelial Cell Matrices Determines Control of Stenosis in a Porcine Femoral Stent Model

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Delivery Site of Perivascular Endothelial Cell Matrices Determines Control of Stenosis in a Porcine Femoral Stent Model

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Abstract

PURPOSE—High restenosis rates are a major limitation of peripheral interventions. Endothelial cells, grown within gelatin matrices and implanted onto the adventitia of injured vessels, inhibit stenosis in experimental models. To determine if this technology could be adapted for minimally invasive procedures, we compared the effects of cells in an implantable sponge to an injectable formulation and investigated the importance of delivery site in a stent model.

MATERIALS AND METHODS—Stents were implanted in the femoral arteries of 30 pigs followed by perivascular implantation of sponges or injection of particles containing allogeneic endothelial cells. Controls received acellular matrices or nothing. The effects of delivery site were assessed by injecting cellular matrices into or adjacent to the perivascular tissue, or into the neighboring muscle. Animals were sacrificed after 28 days. Pre-sacrifice angiograms and tissue sections were evaluated for stenosis.

RESULTS—Arteries treated with cellular matrices had a 55 – 63% decrease in angiographic stenosis ($P<0.05$) and a 38 – 43% reduction ($P<0.05$) in histologic stenoses compared to controls. Intimal area was greatest when cellular matrices were delivered into the muscle (6.35 ± 0.95 mm^2) compared to into or adjacent to the perivascular tissue (4.05 ± 0.56 mm^2 and 4.73 ± 0.53 mm^2, respectively, $P < 0.05$).

CONCLUSIONS—Perivascular endothelial-cell matrices reduced stenosis after stent-induced injury. The effects were not dependent on the formulation but appeared to be dependent upon delivery site. Minimally invasive injections of endothelial-cell matrices to the adventitia of arteries following peripheral interventions may decrease restenosis rates.

Percutaneous revascularization of peripheral atherosclerosis is limited by high rates of failure. Despite high initial technical success, restenosis occurs in approximately 50% of the treated vessels, such as the superficial femoral artery (SFA), within 6–12 months (1–3). While the clinical data for the use of drug eluting stents in the coronary circulation is compelling, a benefit

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of stenting and drug-elution in peripheral arteries has yet to be demonstrated (4–8). A recent small study demonstrated a benefit of paclitaxel-coated angioplasty balloons on restenosis in the femoropopliteal artery (9), however long-term data in large clinical trials of peripheral arterial disease are lacking. New treatment modalities are needed.

Vascular restenosis is a complex response to injury that occurs after all arterial interventions. The combined effects of local injury, thrombosis, inflammation and leukocyte infiltration, spasm, smooth muscle cell proliferation and matrix remodeling progressively destroy the normal architecture and induce a hyperplastic response that encroaches on the blood vessel lumen (10,11). Endothelial cells grown within gelatin matrices and implanted onto the adventitial surfaces of injured blood vessels inhibit stenosis and increase lumen diameter in experimental models of angioplasty, arteriovenous fistula and grafts, controlling the long term effects of intervention in concert with reduction of all phases of the acute response (12–15). The cellular scaffolds are surgically implanted through open fields, exert their peak effects early after implantation and erode thereafter (13,14). What is not yet understood is whether the cellular implants can provide benefit for vascular manipulations when continued applied stress remains after the cellular implant has degraded. Endovascular stents offer a model to examine this question. The stents are expanded in place and retain an outward deforming force against the vessel beyond the peak effect of the cell-seeded scaffolds. Moreover, stent models allow one to address a second logistical issue. Whereas open field surgery is amenable to direct adventitial placement, the question arises as to whether cellular implants could be applied minimally invasively and retain efficacy. In the present study, we investigated both of these issues. We evaluated the vascular response to stent-induced injury in porcine femoral arteries treated with matrices containing allogeneic endothelial cells implanted surgically or injected around the affected vessel or into the adjacent muscle. The last of these groups posed an interesting added question as muscles are often a site of cell or gene implantation and yet their cyclical contraction can impose significant forces on the implanted material. The results of this study provide additional insight into endothelial control after vascular injury.

MATERIALS AND METHODS

Endothelial Cell Culture in Gelatin Matrices

Porcine aortic endothelial cells were isolated from healthy pigs and cultured in gelatin sponges (Gelfoam®) as previously described (15). 4.0 × 1.0 × 0.3 cm blocks of sterile Gelfoam® (Pfizer, New York, NY) were seeded with 1.5×10^5 cells per sponge. Approximately 60–70 mg of gelatin particles (prepared by Pfizer by milling the dry Gelfoam® sponges) were hydrated overnight in media and seeded with cells at a density of 3×10^3 cells per mg particles. The cells were incubated for ≈ 2–3 weeks in Endothelial Basal Media-2 (EBM-2, Lonza, Portsmouth, NH) supplemented with 5% FBS and 50 μg/mL gentamicin. The growth curve was determined by periodic evaluation of cell number after enzymatic digestion (15). Cell viability was assessed by trypan blue exclusion. The cells reached a growth plateau prior to implantation. Functional testing was performed on *in vitro* cohorts. The production of heparan sulfate (HS), transforming growth factor-β1 (TGF-β_1), basic fibroblast growth factor (FGF-2), nitric oxide (NO) and tissue inhibitor of metalloproteinase-2 (TIMP-2) were used as markers of endothelial cell function. Once the cells reached a growth plateau, media was conditioned for 24 hours in EBM supplemented with 0.5% FBS and 50 μg/mL gentamicin, collected, filtered and stored at 4°C or - 80°C until assayed. HS levels in conditioned media were determined using a dimethylmethylene blue binding assay. TGF-β_1 and TIMP-2 concentrations were determined by ELISA (R&D Systems, Minneapolis, MN). Total NO levels were determined indirectly in an ELISA assay (R&D Systems) based on the Greiss Reaction. Control Gelfoam® were incubated for up to 2 weeks in medium containing 5% FBS prior to implantation.

In vivo Biologic Activity of Transplanted Endothelial Cell Matrices

The ability of allogeneic endothelial cell matrices to control vascular repair when placed adjacent to stented porcine femoral arteries was assessed. This study conformed to the guidelines specified in the National Institutes of Health "Guide for Care and Use of Laboratory Animals" and was approved by the Institutional Animal Care and Use Committee of the Harvard Medical School (Boston, MA) and Concord BioMedical Sciences and Emerging Technologies, (Lexington, MA). Thirty male and female domestic pigs, 36.1 kg ± 2.5 kg, were obtained from Animal Biotech, Inc. (Danboro, PA). Anesthesia was induced with Telazol® (4–6 mg/kg, intramuscularly) and nifedipine (10 mg, sublingual) and maintained with inhaled isofluorane (0.5–1.5%) via an endotracheal tube. Buprenorphine (0.01 mg/kg, IM) was also administered. The intra-arterial pressure and electrocardiogram were continuously monitored throughout the procedure. To prevent or reduce the occurrence of thrombotic events, animals were treated on Day - 1 or Day -2 with aspirin (650 mg, *per os* [PO]) and clopidogrel (300 mg, PO). The animals were then treated with aspirin (81 mg, PO) and clopidogrel (75 mg, PO) daily thereafter.

Surgical Procedure

A total of 30 pigs were implanted with stents in the right and left femoral arteries (total stented arteries = 60). Briefly, right carotid arterial access with a 7 French sheath was obtained via cut down, and a 5.0-mm-diameter angioplasty balloon (Abbott Vascular, Redwood City, CA) was advanced to the left and right femoral arteries under fluoroscopic guidance (GE 9800 C-arm fluoroscope, resolution 15 frames per second). The right and left arteries were injured by 30-second balloon inflations at 10 atmospheres pressure (3 inflations per side, in overlapping segments). Biliary stents (Abbott Vascular, 5.0–5.5 mm × 18 mm) were introduced into the left and right femoral arteries. Angiography was performed and the stents were expanded with the 5.0-mm-diameter angioplasty balloon at 10–12 atmospheres pressure. After final angiography to assess vessel patency, the femoral arteries were treated. Two experiments were performed. In the first set of studies the femoral arteries were exposed and an incision made in the perivascular tissue. The arteries were left with no further therapy (sham, n=8 arteries, 2 arteries per animal) or treated with control Gelfoam® sponges (n = 8 arteries, 2 arteries per animal), or Gelfoam® sponges seeded with endothelial cells (n = 10 arteries, 2 arteries per animal) placed directly adjacent to the artery in the space between the perivascular tissue and vessel. In the remaining animals, the femoral arteries were exposed leaving the perivascular tissue intact. Arteries were treated with injections of ≈ 60–70 mg of control Gelfoam® particles (n = 6 arteries, 2 arteries per animal) or Gelfoam® particles seeded with endothelial cells (n = 10 arteries, 2 arteries per animal) in 3 ml media into the space between the perivascular tissue and the artery. In the second experiment, the femoral arteries were exposed and the arteries treated by injecting ≈ 60–70 mg in 3 ml media of Gelfoam® particles seeded with endothelial cells into the space between the perivascular tissue and the artery (n = 6, 2 arteries per animal) or outside the perivascular tissue (n = 6, 2 arteries per animal). In the last treatment group, the adjacent muscle was exposed and the cellular particles were injected into the adjacent muscle capsule (n = 6, 2 arteries per animal). All injections of particles were performed using a 20 gauge needle and syringe. Heparin (50–200 U/kg, IV) was administered to prolong activated clotting time (ACT) to a target range of ≈ 275 seconds during stent deployment. ACT was monitored and additional heparin was administered as needed. Animals were euthanized on day 28 and the femoral arteries plus stent were processed for histological evaluation.

Quantitative Vascular Angiography

For each artery, angiography was performed prior to injury, at the time of stent deployment, immediately after deployment and on day 28. Quantitative angiography was performed on recorded images using Medcon Limited (Medcon Telemedicine Technology Whippany, NJ)

customized software in a blinded fashion. Measurements (mm) of arterial diameters were made pre-injury (Artery), during stent deployment (Deploy) and post –stent placement (After). The minimum lumen diameter (MLD) was measured on the pre-sacrifice angiogram. In the first experiment, acute luminal gain (After – Artery) and late lumen loss (After – MLD) were calculated. For both experiments, measurements were made of the reference unstented vessel (ref) and MLD on day 28 and angiographic stenosis calculated as follows: (ref vessel – MLD)/(ref vessel) × 100. Balloon/artery ratios were calculated (Deploy/Artery) to determine the extent of injury for each treatment group (16).

Tissue Processing

On the 28th post-operative days, animals were euthanized with intravenous potassium chloride (40 mEq). The femoral arteries were perfused at 100 mm Hg with Ringer's lactate solution followed by 10% neutral formalin to fix the arteries in situ. The arteries were isolated and the vessel divided into five 10-mm long segments: far proximal to the stent (1–3 mm upstream from the stent), proximal stent, middle of the stent, distal stent and far distal to the stent (1–3 mm beyond the stent). The stented segments were methacrylate embedded and non-stented vessel segments were paraffin embedded. 5-μm sections were obtained and stained with hematoxylin and eosin and Verhoeff's elastin stain. Slides were read and interpreted by a board-certified veterinary pathologist blinded as to treatment groups. Histomorphologic findings were graded on a scale from 1 through 3, depending upon severity (17) (0 = Absent (no finding/response); 1 = Present, but minimal feature (i.e., a finding of minimal magnitude/distribution with no anticipated adverse effect on local tissue function or viability); 2 = Notable feature (i.e., a finding of mild to moderate magnitude/distribution which may adversely affect local tissue function or viability); 3 = Overwhelming feature (i.e., a finding of marked/severe magnitude/distribution which likely adversely effects local tissue function or viability). Histomorphometric analysis was performed on all segments. The intimal (I), medial (M) and lumen (L) areas were measured using computerized digital planimetry with a video microscope and customized software. Comparisons were made between the average intimal area or intimal thickness, the average % stenosis and the average worst % stenosis. The % stenosis is defined as the intimal area divided by the intimal area plus the lumen area (I/I+L). The average % stenosis is the average of the three segments (proximal, middle and distal) for each vessel. The worst % stenosis is the maximum % stenosis of either the proximal, middle or distal segment for each vessel.

Statistical Analysis

All data are presented as mean ± SE. Statistical analysis comparing treatment groups used a single factor ANOVA and a non-paired Students t-test. Values of $P<0.05$ were considered significant.

RESULTS

Biochemical Activity of Endothelial Cells Cultured within Gelatin Matrices

Endothelial cells within Gelfoam® sponges or particles were assayed for cell number, viability, TGF-β_1, HS, FGF-2, NO and TIMP-2 production. The cells cultured within sponges lined the interstices of the three-dimensional matrix and followed a growth pattern similar to that observed for cells cultured on tissue culture dishes. Viability remained ≥ 90% during the 2–3 week culture course. Cells cultured on Gelfoam® particles displayed similar growth kinetics and viability. Conditioned media was prepared from *in vitro* cohorts of sponges and particles embedded with endothelial cells. HS, TIMP-2, NO, TGF-β_1 and FGF-2 were detected in conditioned media with similar levels for cells cultured within sponges or on the particles (Table 1).

Efficacy of Perivascular Endothelial Cell Matrices

All animals survived the interventional procedures and cell placement until tissue harvest at 28- days. All incisions healed well and all animals gained weight throughout the post-operative period. All of the femoral arteries were patent at the 28 day time point. Angiographic analysis performed on pigs four weeks post injury revealed stenosis in the stented segments of control animals (Figure 1, Table 2). The % stenosis by angiography of control animals that received Gelfoam® sponges (20.1 ± 3.5%), Gelfoam® particles (17.4 ± 3.7%) or sham (18.1 ± 1.9%) did not differ significantly. Application of cell seeded Gelfoam® sponges reduced % stenosis of the stented arteries by 63% ($P<0.05$) compared to Gelfoam® controls, to 7.41 ± 1.4% stenosis (Table 2). Similarly, compared to Gelfoam® controls, cell seeded Gelfoam® particles reduced the % stenosis by 55% ($P<0.05$) to 7.8 ± 1.2%. Morphometic analysis revealed similar results (Figure 1). Treatment with Gelfoam® sponges embedded with endothelial cells reduced the intimal area from 3.50 ± 0.55 and 2.50 ± 0.25 for sham and Gelfoam® controls, respectively, to 1.85 ± 0.30 ($P<0.05$) (Table 3). The cellular matrices also significantly decreased the average and worst % stenosis compared to Gelfoam® and sham controls ($P<0.05$). Treatment of arteries with cell seeded Gelfoam® particles resulted in a significant increase in the lumen area from 10.90 ± 0.92 and 12.42 ± 0.70 for sham and Gelfoam controls, respectively, to 14.2 ± 0.48 ($P<0.05$). Cell seeded particles also significantly reduced the average and worst % stenosis compared to Gelfoam® and sham controls ($P<0.05$) (Table 3).

Effects of Endothelial Cell Delivery Site

The pigs used in this study received endothelial cells in Gelfoam® particles injected at one of the following treatment sites: into the space between the perivascular tissue and artery, injected adjacent to the perivascular tissue or into the adjacent muscle capsule (Figure 2). All incisions healed well and all animals gained weight throughout the post-operative period. All of the femoral arteries were patent at the four week time point. Detailed angiographic analysis at four weeks post injury revealed an increase in stenosis in the stented segments of the vessels in animals that received injections into the adjacent muscle capsule compared to those who received injections into or adjacent to the perivascular tissue (Figure 3). Arteries treated with endothelial cell matrices injected into or adjacent to the perivascular tissue had a % stenosis of 17.2 ± 4.1 and 13.9 ± 2.5, respectively. In comparison, arteries treated with endothelial cell matrices injected into the adjacent muscle had a % stenosis of 26.7 ± 3.5 ($P<0.05$). The area of maximal stenosis identified angiographically agreed with that observed upon histologic assessment and corresponded to the proximal segment of the stented vessels. Analysis of the proximal planes, where the greatest response appeared to be present, also revealed significantly greater intimal area ($P<0.05$) and thickness in animals injected with endothelial cell matrices into the adjacent muscle (Figure 3, Table 4) compared to those who received injections of endothelial cell matrices into or on top of the perivascular tissue. However, there was no statistical difference between the groups who received injections of endothelial cell matrices into or on top of the perivascular tissue.

The levels of inflammation and fibrin deposition associated with the lumen were scored and found to be low in all treatment groups evaluated (Table 5). Inflammation scores were semi-quantitative measures of the extent of local arterial wall inflammation and each stent strut was scored according to the degree of inflammation. Fibrin deposition in the intima is a characteristic response seen in drug-eluting stents and is not expected with bare metal stents. All treatment groups exhibited similar levels of fibrin deposition and were representative of bare metal stents at 4 weeks. The inflammatory and fibrotic response associated with the adventitia, perivascular tissue and muscle were also evaluated (Table 5). Overall, there was very little perivascular inflammatory response. The majority of the inflammatory cell types associated with the adventitia and perivascular tissue consisted of histocytes and lymphocytes. Arteries treated with injections of endothelial cell matrices adjacent to the perivascular tissue

had the least amount of lymphocytes in the adventitia and perivascular tissue when compared to the other groups. Adventitial fibrosis was also present in all treatment groups and was generally minimal, however, adventitial fibrosis tended to be the least severe in arteries treated with injections of endothelial cell matrices adjacent to the perivascular tissue.

DISCUSSION

Peripheral vascular disease affects greater than 5 million adults in the United States (18) and is both an important clinical challenge and a critical model in which to examine aspects of vascular repair. Restenosis is still a significant limitation of most arterial interventions performed in the peripheral circulation. In particular, interventional treatments in the SFA have long suffered from excessively high restenosis rates regardless of treatment with balloon angioplasty and stenting (5,19). The poor performance of stents and the complexity of disease in the SFA have prompted the development of new technologies and techniques to attempt to address this problem (20,21).

The tissue engineered endothelial technology presented here represents a novel approach to the treatment and study of complications associated with peripheral interventions. When placed perivascularly, endothelial cells embedded within matrices regulate the response to vascular injury and decrease stenoses and negative vascular remodeling (12–15,22). However, all of these studies utilized a porous, three-dimensional Gelfoam® sponge as the support matrix for implantation of quiescent and therapeutic endothelial cells. The application of this technology was therefore limited to those that required an open, surgical procedure such as the creation of AVG or AVF for hemodialysis access or bypass grafts (23). An aim of the present study was to determine if this technology could be adapted for minimally invasive procedures and if such an injectable formulation would have similar efficacy and benefits in vivo. Endothelial cells were grown within Gelfoam® sponges or Gelfoam® particles, which is prepared by milling the sponges and therefore represents the same material. The cells displayed similar growth curves whether grown within sponges or in suspension on the particles (15,22,24). More importantly, the levels of HS, TGF-β_1, FGF-2, NO and TIMP-2 in the media conditioned by endothelial cells grown within sponges are similar to that from cells grown in the particles, suggesting that both types of matrices support the quiescent and therapeutic endothelial phenotype (25). This was confirmed *in vivo* by the comparison of the implantable sponge and injectable particle formulation in a porcine femoral stent model. Both Gelfoam® sponges and particles seeded with endothelial cells significantly inhibited in-stent stenosis observed angiographically and morphometrically. Injections of endothelial cell seeded Gelfoam® particles appeared to provide the greatest benefit when the cellular matrices were delivered either into or adjacent to the perivascular tissue. However, the beneficial effects appeared to be lost when the injections were into the adjacent muscle capsule and further away from the vessel. This may be due to the physical distance which separated the cellular matrices from the affected vessel and decreased the bioavailability of the various endothelial-derived therapeutic molecules. It may also be due in part to potential adverse effects of the muscle microenvironment on the viability of the endothelial cells in the cellular matrix, which may decrease the production and release of the therapeutic molecules. Indeed, some endothelial-derived molecules, such as nitric oxide, can only exert their beneficial effects in close proximity due to a short half-life (26) and charged molecules such as heparan sulfate and matrix-binding cytokines such as TGF-β_1 mostly exert their biological effects in the local environment where they are produced due to their lack of diffusion in tissue (27–29). Furthermore, the extracellular matrix-rich membrane of the muscle capsule likely limited the diffusion and effects of endothelial cell- secreted molecules to the affected vessels (30). The cyclical contraction of the muscle can also impose significant forces on the implanted material affecting cell viability. It has been reported that injection of various cell types into the contractile muscle tissue of the heart resulted in poor cell survival in part due to the unfavorable relatively hypoxic microenvironment (31). It is

therefore reasonable that the injection of endothelial cell matrices inside the muscle capsule could also result in decreased cell survival due to hypoxia compared to injection closer to the vessel where in particular oxygen levels are higher. The results of the present study suggest that perivascular endothelial cell matrices promote vascular repair after stent-induced injury and delivery of cellular matrices adjacent to the perivascular tissue provided similar benefit to delivery into the perivascular tissue. Moreover, cellular matrices delivered outside the perivascular tissue appeared to have a lower incidence of adventitial lymphocytes and fibrosis compared to cellular matrices delivered inside the perivascular tissue, although they were equally effective in suppressing intimal formation and stenosis.

A limitation of the current study was that an open surgical procedure was used for all deliveries; however this was intentional as confirmation of exact delivery site was desired as well as direct comparison of the sponge and particle formulations independent of procedural delivery. Follow-up studies will need to be performed to evaluate the long term biological effects of the injectable formulation delivered during a minimally invasive procedure, either by catheter or percutaneous injection. The animals used in this study were healthy and therefore the impact of stenting an existing lesion was not assessed. However, the data presented here suggests that delivery of perivascular endothelial cell matrices co-incident with balloon angioplasty or stenting of peripheral arteries may improve the long-term success rates of these procedures. Perivascular endothelial cell matrices controlled the response to endovascular stent injury when applied via direct open field implantation or injection, but were less effective if injected into local contracting skeletal muscle beds. These findings offer further insight into the biology of endothelial control of vascular healing, and expand the opportunity for the clinical use of cell based therapies.

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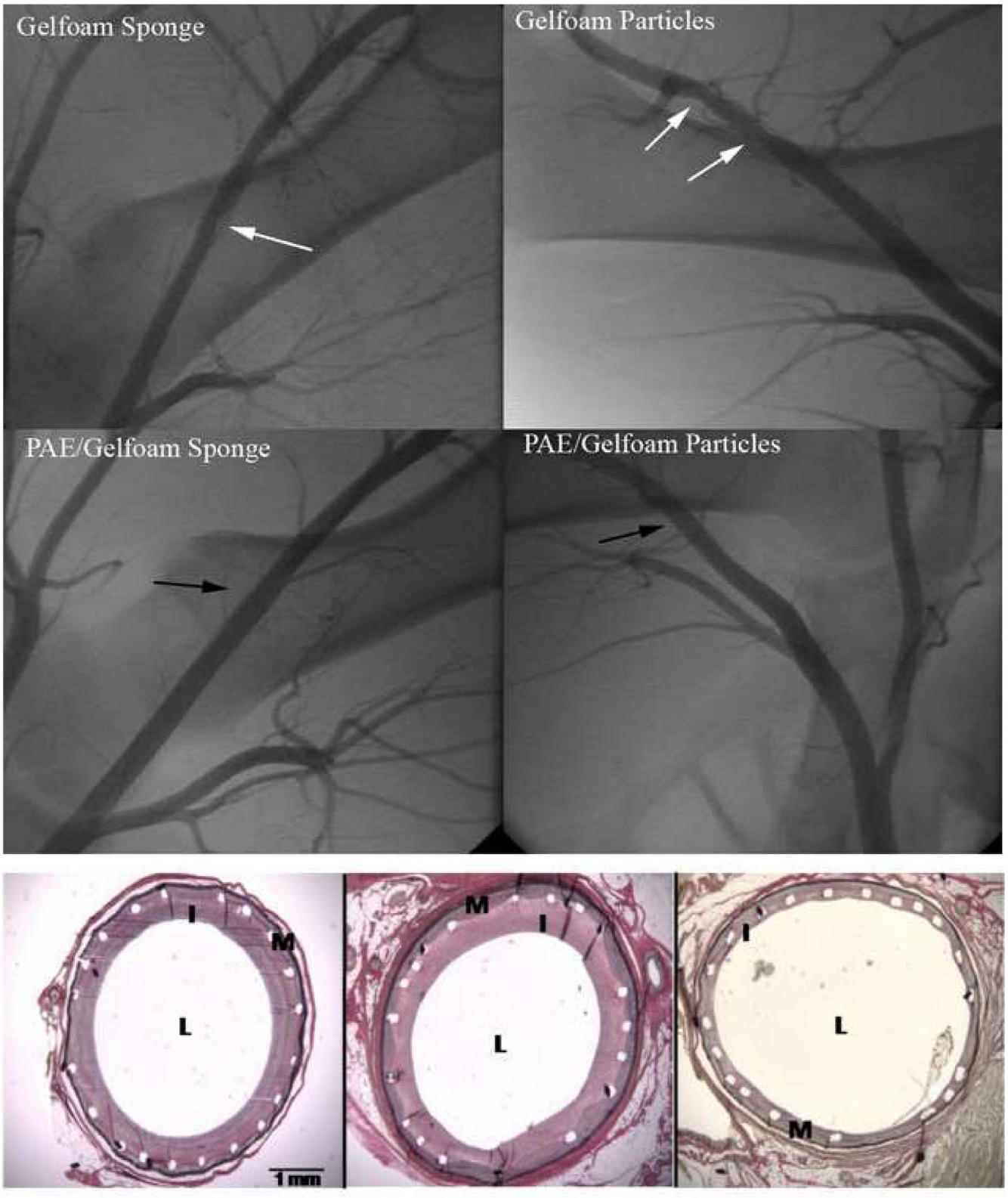

Figure 1.

Representative day 28 angiograms (A) and photomicrographs of Verhoeff's elastin stained arterial cross sections (B) from the efficacy analysis. Comparison of the angiograms show significant stenosis in the stented region of control arteries (white arrows) compared to arteries treated with cell containing sponges or particles (black arrows). Histological sections show significantly greater intimal area in control sham (left panel) and Gelfoam® (middle panel) arteries compared to arteries treated with perivascular endothelial cell matrices (right panel). I = Intima, M = Media, L = Lumen.

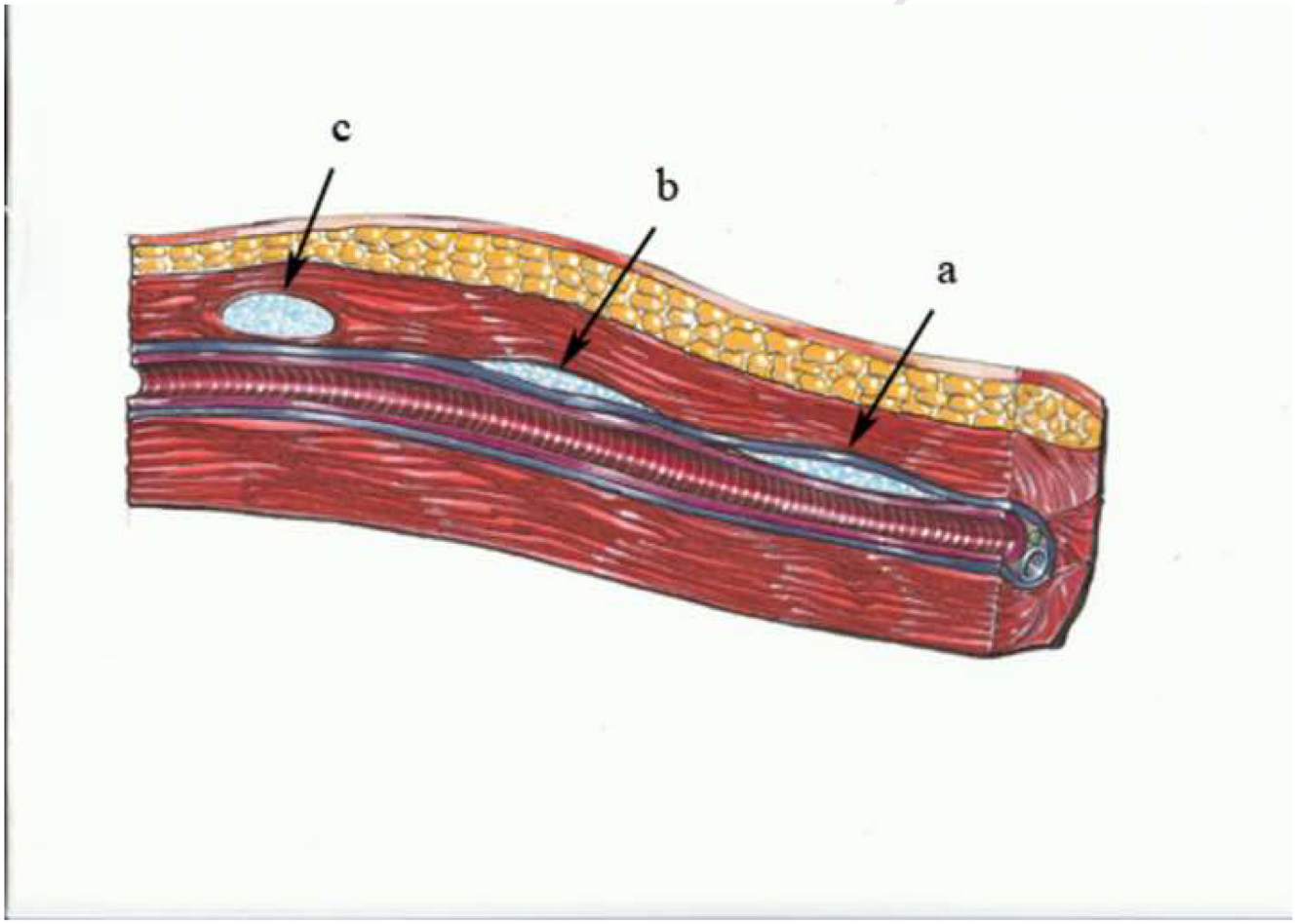

Figure 2.
Diagram shows particle injection sites relative to the femoral artery. Injections of particles into the perivascular tissue (a), adjacent to the perivascular tissue (b) and into the muscle capsule (c).

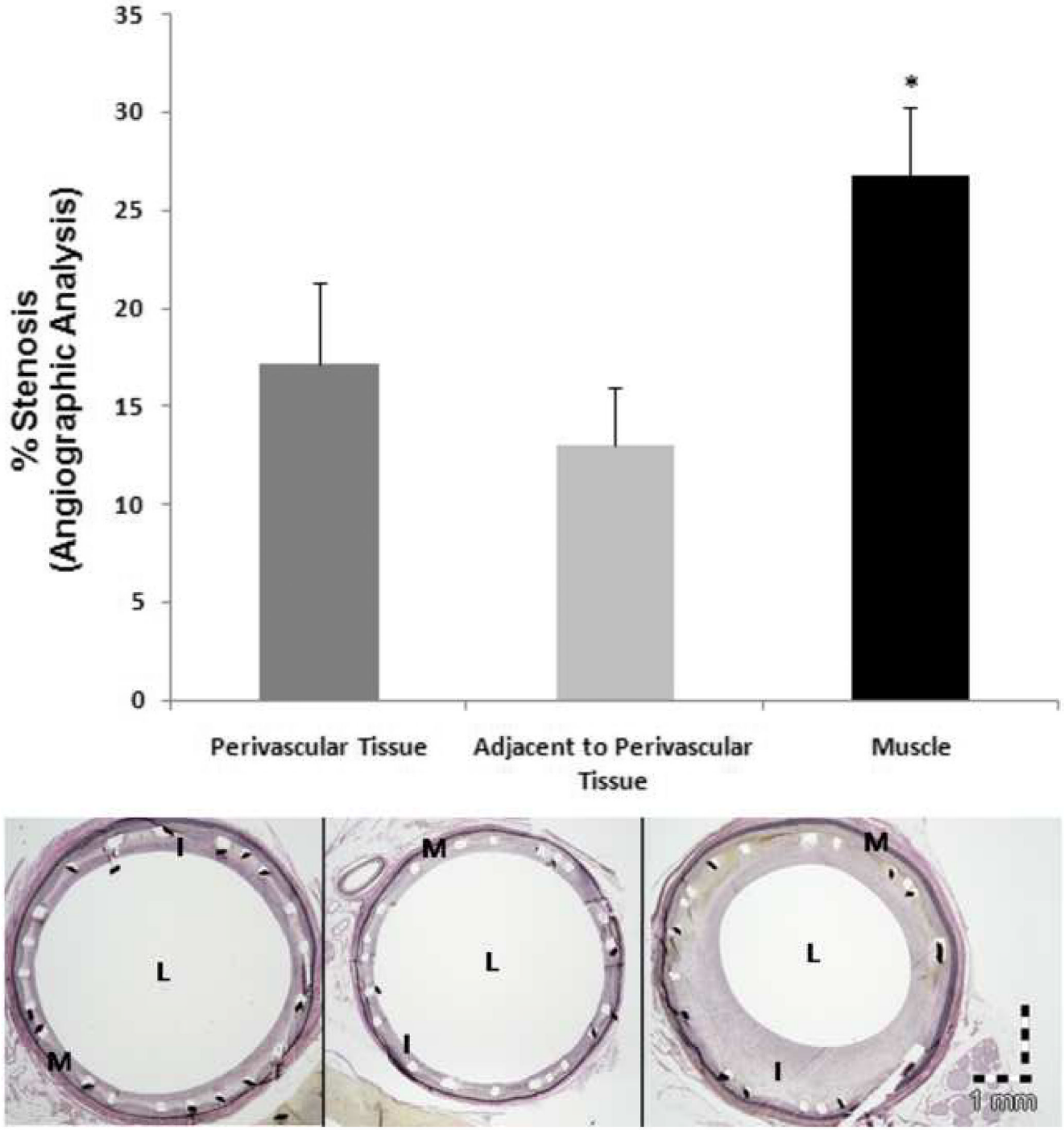

Figure 3.

Representative day 28 angiographic measurements (A) and photomicrographs of Verhoeff elastin stained arterial cross sections (B) from the injection site analysis. Bar graph shows the average percentage of stenosis as a function of injection site. **P*<0.05 compared to the other two groups. Greater intimal formation was observed for arteries treated with cellular matrices injected into the muscle capsule (B, right panel) compared to arteries treated with cellular

matrices injected into or adjacent to the perivascular tissue (B, left and middle panels, respectively). I = Intima, M = Media, L = Lumen.

Table 1

Functional Analysis of Endothelial Cells within Gelatin Matrices (*in vitro* cohorts)

Assay	PAE/Gelfoam® Particles (≈60 mg)	PAE/Gelfoam® Sponge (1 × 4 × 0.3 cm)
Cell Count	$2.0 \pm 0.53 \times 10^6$	$1.36 \pm 0.08 \times 10^6$
Viability	94 ± 1.2 %	92 ± 1.9 %
μg/mL HS	0.72 ± 0.14	1.07 ± 0.15
pg/mL TGF-β_1	482 ± 139	466 ± 32
pg/mL FGF-2	106 ± 36	166 ± 33
μM NO	4.9 ± 0.42	2.03 ± 0.30
ng/mL TIMP-2	14.7 ± 0.28	10.5 ± 1.33

PAE = porcine aortic endothelial cells; HS = heparan sulfate; TGF-β_1 = transforming growth factor - β_1; FGF-2 = fibroblast growth factor-2; NO = nitric oxide; TIMP-2 = tissue inhibitor of metalloproteinase-2

TABLE 2

Quantitative Angiography of Porcine Stented Femoral Arteries – Efficacy Analysis

Treatment	Artery (mm)*	B/A Ratio†	Acute Gain (mm)‡	28d MLD (mm)§	Late Loss (mm)‖	% Stenosis#
Gel/Sponge	6.27 ± 0.14	1.13 ± 0.02	0.63 ± 0.08	4.11 ± 0.28	2.79 ± 0.25	20.0 ± 3.5
PAE/Gel Sponge	5.68 ± 0.29	1.17 ± 0.06	0.76 ± 0.42	5.0 ± 0.10**	1.44 ± 0.26**	7.41 ± 1.4**
Sham	5.96 ± 0.37	1.27 ± 0.06	0.98 ± 0.24	4.32 ± 0.21	2.62 ± 0.27	18.1 ± 1.9
Gel/Injected	6.61 ± 0.23	1.08 ± 0.02	0.32 ± 0.05	4.53 ± 0.48	2.40 ± 0.42	17.4 ± 3.7
PAE/Gel Injected	5.96 ± 0.27	1.25 ± 0.07	1.11 ± 0.29	5.50 ± 0.19**	1.58 ± 0.19**	7.8 ± 1.2**

*Artery = pre-injury;

†B/A = Balloon/Artery;

‡Acute Gain = (After – Artery);

§MLD = Minimum Lumen Diameter

‖Late Loss = (After – MLD);

#%Stenosis = (ref vessel – MLD)/(ref vessel) × 100;

**P< 0.05 compared to control arteries Gel = Gelfoam®;

PAE = porcine aortic endothelial cells

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TABLE 3

Histopathological Characteristics of Stented Porcine Femoral Arteries – Efficacy Analysis

Characteristics	Control Gelfoam® Sponges	PAE/Gelfoam® Sponges	Sham	Control Gelfoam® Particles	PAE/Gelfoam® Particles
Number of arteries, *n*	8	10	8	6	10
Intima Area (mm^2)	2.50 ± 0.35	1.85 ± 0.30*	3.50 ± 0.55	3.64 ± 0.66	2.35 ± 0.27
Media Area (mm^2)	2.05 ± 0.11	2.20 ± 0.17	2.17 ± 0.25	3.09 ± 0.08†	2.60 ± 0.24
Lumen Area (mm^2)	8.60 ± 0.48†	10.48 ± 0.48	10.90 ± 0.92	12.42 ± 0.70	14.2 ± 0.48*
Average % Stenosis‡	24.0 ± 2.6	15.7 ± 1.9*	25.2 ± 3.6	23.0 ± 4.1	14.4 ± 1.5*
Worst % Stenosis‡	38 ± 5.6	22.5 ± 3.1*	33 ± 4.7	33 ± 6.5	20.0 ± 2.1*

*$P < 0.05$ compared to control arteries;

†$P < 0.05$ compared to PAE and Sham;

‡% Stenosis = (I/I+L) × 100

PAE = porcine aortic endothelial cells

TABLE 4

Histopathological Characteristics of Stented Porcine Femoral Arteries - Injection Site Analysis

Characteristics	PAE/Gel Injected Into Perivascular Tissue	PAE/Gel Injected Adjacent to Perivascular Tissue	PAE/Gel Injected Into Adjacent Muscle
Number of arteries, *n*	6	6	6
Intima Area (mm^2)	3.50 ± 0.40	3.85 ± 0.22	4.68 ± 0.45
• Proximal	4.05 ± 0.56	4.75 ± 0.53	6.35 ± 0.95*
Intimal Thickness (mm)	0.27 ± 0.08	0.27 ± 0.03	0.35 ± 0.10
• Proximal	0.31 ± 0.12	0.33 ± 0.08	0.46 ± 0.20
Media Area (mm^2)	2.21 ± 0.14	2.20 ± 0.12	2.38 ± 0.16
• Proximal	2.49 ± 0.33	2.35 ± 0.18	2.56 ± 0.13
Lumen Area (mm^2)	12.51 ± 0.67	14.5 ± 0.63	12.58 ± 1.11
• Proximal	12.38 ± 0.91	13.90 ± 0.34	12.57 ± 1.17
Average % Stenosis†	22.0 ± 2.5	21.0 ± 0.41	28.0 ± 3.3
• Proximal	25 ± 3.70	25 ± 2.05	34 ± 5.33

*P< 0.05 compared to other treatment groups;

†% Stenosis = (I/I+L) × 100

PAE = porcine aortic endothelial cells; Gel = Gelfoam®

TABLE 5

Inflammatory* and Fibrotic† Response – Injection Site Analysis

Characteristic	PAE/Gel - Into Perivascular Tissue	PAE/Gel - Adjacent to Perivascular Tissue	PAE/Gel - Adjacent Muscle	Control Gelfoam (Into Perivascular Tissue)
Intimal Inflammation	1.0 ± 0.08	1.02 ± 0.06	1.03 ± 0.05	1.02 ± 0.11
Intimal Fibrin	0.56 ± 0.21	0.67 ± 0.12	1.0 ± 0.29	0.72 ± 0.18
Adventitial Inflammation:				
Lymphocytes	0.72 ± 0.20	0.39 ± 0.20	0.61± 0.21	1.00 ± 0.28
Histocytes	0.78 ± 0.18	0.89 ± 0.07	0.89 ± 0.07	0.89 ± 0.25
Perivascular tissue Inflammation:				
Lymphocytes	1.06 ± 0.32	0.00	0.11 ± 0.11	0.67 ± 0.44
Histocytes	0.72 ± 0.15	0.00	0.06 ± 0.06	0.72 ± 0.46
Giant Cells	0.28 ± 0.20	0.00	0.00	0.17 ± 0.17
Granulomatous Reaction	0.00	0.00	0.00	0.33 ± 0.33
Adventitial Fibrosis	1.50 ± 0.14	0.67 ± 0.09	1.11 ± 0.16	1.44 ± 0.28

* All cellular infiltrates were identified by their typical microscopic morphology

† Fibrin was identified as interstitial accumulations of amorphous and acellular eosinophilic material

PAE = porcine aortic endothelial cells; Gel = Gelfoam®