Attachment of Human Endothelial Cells to Polyester Vascular Grafts: Pre-Coating With Adhesive Protein Assemblies and Resistance to Short-Term Shear Stress

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Summary
Cardiovascular prosthetic bypass grafts do not endothelialize spontaneously in humans, and so they pose a thrombotic risk. Seeding with cells improves their performance, particularly in small-caliber applications. Knitted tubular polyethylene-terephthalate (PET) vascular prostheses (6 mm) with commercial type I collagen (PET/Co) were modified in the lumen by the adsorption of laminin (LM), by coating with a fibrin network (Fb) or a combination of Fb and fibronectin (Fb/FN). Primary human saphenous vein endothelial cells were seeded (1.50 × 10^5/cm²), cultured for 72 h and exposed to laminar shear stress 15 dyn/cm² for 40 and 120 min. The control static grafts were excluded from shearing. The cell adherence after 4 h on PET/Co, PET/Co +LM, PET/Co +Fb and PET/Co +Fb/FN was 22 %, 30 %, 19 % and 27 % of seeding, respectively. Compared to the static grafts, the cell density on PET/Co and PET/Co +LM dropped to 61 % and 50 %, respectively, after 120 min of flow. The cells on PET/Co +Fb and PET/Co +Fb/FN did not show any detachment during 2 h of shear stress. Pre-coating the clinically-used PET/Co vascular prosthesis with LM or Fb/FN adhesive protein assemblies promotes the adherence of endothelium. Cell retention under flow is improved particularly on fibrin-containing (Fb and Fb/FN) surfaces.

Key words
Blood vessel prosthesis • Polyethylene terephthalate • Endothelial cells • Fibrin • Shear stress

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Introduction
Atherosclerosis is one of the most common diseases in the developed countries. Surgical intervention in the form of a bypass or blood vessel substitution is often required to restore the blood supply to the diseased heart, brain, extremities or inner organs (Balaz et al. 2009). Autologous (patient’s own) artery or vein is an ideal natural material for bypass graft procedures, but it is often unavailable or unsuitable for reconstruction (Matia et al. 2007, Kachlík et al. 2008). Vascular prostheses made of non-degradable synthetic polymers are therefore widely used in cardiovascular surgery, namely expanded polytetrafluoroethylene (ePTFE, Teflon, Gore-Tex) or polyethylene terephthalate (PET, Dacron). Although they perform well as substitutes for large-caliber blood...
vessels, they fail in small-caliber applications (≤ 6 mm), e.g. in coronary, below-the-knee or micro-vascular regions (Kannan et al. 2005, Chlupác et al. 2009, Rémy et al. 2013).

The healing process of synthetic vascular prostheses is incomplete in humans. It comprises the formation of a fibrous capsule around the outer surface, minimal capillary in-growth into the fiber interstices, and no lining or sparse lining with endothelial cells (EC) in the lumen (Pasquinelli et al. 1990). Thus, the blood-contacting surface remains unhealed and covered with a layer of compacted plasma proteins only. Synthetic vascular grafts therefore fail, in part due to thrombosis resulting from the lack of endothelium (Zilla et al. 2007). Moreover, the mismatch in mechanical properties between the elastic native artery and the rigid prosthetic graft leads to proliferation (intimal hyperplasia – stenosis) of vascular smooth muscle cells (VSMC) which cannot be regulated by missing EC (Sarkar et al. 2006, Varga et al. 2013). Bio-functionalization of vascular prostheses and subsequent in vitro lining with EC was previously shown to reduce the risk of thrombosis and graft failure and to improve the clinical performance of vascular prostheses (Vara et al. 2005, Bordenave et al. 2008, Deutsch et al. 2009).

There is no significant difference in long-term patency between PET and ePTFE bypass grafts (Roll et al. 2008), and the choice between PET and ePTFE is usually left to the discretion of the implanting surgeon; however, some vascular specialists prefer PET over ePTFE because PET has better handling characteristics, and there is less bleeding from suture holes and a lower tendency to form peri-graft seroma (Jonas et al. 1987).

The objectives of our study were to modify the inner surface of clinically-used collagen type I-impregnated PET vascular prostheses with immobilization of LM (Engelbreth-Holm-Swarm Murine sarcoma, Sigma) 40 μg/ml in phosphate-buffered saline (PBS) overnight or by a Fb network coating prepared by activation of human fibrinogen (Fbg) (Sigma) with surface-attached thrombin (Sigma) according to a previously-published method (Riedel et al. 2009). Briefly, Fbg (50 μg/ml in Tris buffer (TB) for 60 min) was adsorbed on the surface of the graft, rinsed with TB, followed by thrombin (2.5 NIH U/ml in TB for 10 min), TB, and Fbg (200 μg/ml in TB containing antithrombin III (Chromogenix) (0.5 U/ml) for 2 h. A surface fibrin network was formed by catalytic action of the surface-attached thrombin on the ambient fibrinogen solution. In the third sample, the fibrin network was further coated with FN (human plasma, Roche) 50 μg/ml in PBS overnight. An unmodified (commercial) PET/Co prosthesis served as a control graft. Thus, 4 graft types were prepared: PET/Co, PET/Co +LM, PET/Co +Fb and PET/Co +Fb/FN (Institute of Macromolecular Chemistry, Academy of Sciences of the Czech Republic, v.v.i.). They were all immersed for 12 h into M199 standard culture medium (Invitrogen).

The prostheses were cut into pieces 20 cm in length and modified on the inner surface by immobilization of LM (Engelbreth-Holm-Swarm Murine sarcoma, Sigma) 40 μg/ml in phosphate-buffered saline (PBS) overnight or by a Fb network coating prepared by activation of human fibrinogen (Fbg) (Sigma) with surface-attached thrombin (Sigma) according to a previously-published method (Riedel et al. 2009). Briefly, Fbg (50 μg/ml in Tris buffer (TB) for 60 min) was adsorbed on the surface of the graft, rinsed with TB, followed by thrombin (2.5 NIH U/ml in TB for 10 min), TB, and Fbg (200 μg/ml in TB containing antithrombin III (Chromogenix) (0.5 U/ml) for 2 h. A surface fibrin network was formed by catalytic action of the surface-attached thrombin on the ambient fibrinogen solution. In the third sample, the fibrin network was further coated with FN (human plasma, Roche) 50 μg/ml in PBS overnight. An unmodified (commercial) PET/Co prosthesis served as a control graft. Thus, 4 graft types were prepared: PET/Co, PET/Co +LM, PET/Co +Fb and PET/Co +Fb/FN (Institute of Macromolecular Chemistry, Academy of Sciences of the Czech Republic, v.v.i.). They were all immersed for 12 h into M199 standard culture medium (Invitrogen).

Human saphenous vein endothelial cells (HSVEC) were enzymatically harvested from vein remnants (according to Fernandez et al. 2001) obtained from patients undergoing cardiac bypass surgery.
(Université Victor Segalen, Bordeaux, France), cultured in polystyrene flasks (Falcon, 75 cm²) and amplified to passage number P3-5. The procedure was approved by the local Ethics Committee. The culture medium consisted of M199, 20% of fetal calf serum (FCS) (PAA), heparin 50 IU/ml (Choay), basic fibroblast growth factor b-FGF 10 ng/ml (Promocell) and a mixture solution of penicillin (10 × 10³ IU/ml), streptomycin (10 µg/ml) (Sigma) and amphotericin B (250 µg/ml) (Invitrogen). The cellular suspension was homogenized in a fresh medium and injected into the lumen of the prosthesis. Both ends were ligatured and the grafts were placed into a rotating device (Endostrabilisator, Biegler Co. Ltd., Austria) for 4 h (at 5 rpm) to achieve homogeneous cell seeding. The initial seeding density was about 1.50 × 10⁵ cells/cm². The seeding was followed by 3 days of maturation in culture flasks with a fresh complete medium without rotation in a humid incubator at 37 °C with 5% CO₂.

The flow experiment was performed in a custom-built hemodynamic chamber (Inserm U577, Université Victor Segalen, Bordeaux, France) (Fig. 2) consisting of a silicon tubing circuit (Tygon, Masterflex), a peristaltic pump (Cobe, Stöckert, Germany), a water container with a heater (Julabo, Labortechnik Ltd., Germany), a flowmeter (EMKA Technologies, Transsonic Systems Inc., USA), and a tensiometer (Stirecst 960, Siemens).

![Fig. 2. Hemodynamic bioreactor consisting of a circuit of silicon tubing (A), a peristaltic pump (B), a water container with a heater (C), a static control prosthesis that is located in a blind branch (D), a dynamic tested prosthesis that is exposed to flow (E) and a flow-meter (F) (Inserm U577, Université Victor Segalen, Bordeaux, France).](image)

The prosthesis was mounted on a special metal connector creating laminar flow, and the dynamic conditions inside the bioreactor simulated human arterial circulation for 40 and 120 min (temperature 37 °C, flow 500 ml/min, pulsatile pressure 120/60 mm Hg, laminar shear stress (SS) 15 dynes/cm² and medium viscosity 0.04 Poiseuille – achieved by adding 8% Dextran (Sigma, D4751, MW 68 800). FCS (10%) was added to the medium in the 120-min experiment. A static control prosthesis was placed into a blind branch of the circuit (Fig. 2D) and was thus submitted to the same conditions of medium pressure and temperature but excluding flow and shear stress.

After each step of the experiment (seeding – maturation – flow) a prosthesis ring of defined surface area was cut open, washed with PBS and stained with the immunofluorescent Live/Dead Viability/Cytotoxicity Kit (Molecular Probes) to visualize the homogeneity and the efficiency of the cell adhesion and retention (Olympus IX50 fluorescence microscope and Olympus DP70 digital camera, magnification 10 ×). The cells were then removed from the support using a PBS solution with 0.125% trypsin (Sigma) and 0.0625% EDTA (T/E, Sigma). M199 medium with 10% FCS was used to stop the action of the T/E solution. The cells were counted in a 1 mm³ Malassez chamber (8 counts) to obtain the cell number and to calculate the cell population density. The surface of the prosthesis was observed again in the fluorescence microscope to check that it was really devoid of cells after trypsinization. The cell densities on the flow-exposed prostheses were compared to the corresponding static grafts to evaluate the cell detachment during flow. Pieces of the seeded prostheses were also stained with mouse anti-human-β-actin IgG1 (1:100) monoclonal antibody (Sigma) or rabbit anti-human-von Willebrand Factor IgG (1:100) monoclonal antibody (Sigma), and the cell nuclei were counterstained with propidium iodide (5 µg/ml) (Sigma). The morphology of the cells was then evaluated with confocal microscopy (Leica TCS SP2 AOBS) at magnification 10 ×.

**Statistical analysis**

The data is expressed as mean and standard deviation (SD) from 4 to 16 counts. The experiments on PET/Co and PET/Co +Fb (40 min) were run in duplicate and the data was pooled. One-way ANOVA multivariate analysis (Student-Newman-Keuls test) was applied for a statistical evaluation (SigmaStat 3.1 2004, Systat Software, Inc.). Statistical significance was considered when the probability of error was p≤0.05.

**Results**

The adherence of HSVEC to the PET vascular prostheses is shown in Figure 3A. The cell densities 4 h
after rotational seeding amounted to 22 % of the initial seeding density on PET/Co, 30 % of the initial seeding density on PET/Co +LM, a value of 19 % on PET/Co +Fb, and a value of 27 % on PET/Co +Fb/FN. Thus, the cell adhesion and seeding efficiency was better on the vascular prostheses coated with LM (p<0.001) and Fb/FN (p<0.05) than on the control PET/Co prosthesis. Further differences were detected among the coated grafts – coating with LM resulted in significantly better adhesion than coating with Fb (p<0.001) or with Fb/FN (p<0.05). Deposition of Fb resulted in cell density similar to the control graft, but adding FN to the Fb gel (PET/Co +Fb/FN) enhanced the cell adherence (p<0.01).

Fig. 3. A. Adherence of human saphenous vein endothelial cells to PET vascular prostheses 4 h after seeding 1.50 × 10^5 cells/cm². The percentage of initial seeding density is shown at the bottom of each column. B. Flow-resistance of human saphenous vein endothelial cells on PET vascular prostheses. The laminar shear stress was 15 dyn/cm². The statistical differences are given versus the corresponding static control (= 100 %), which was left without shearing (white columns). Data are presented as mean ± standard deviation (SD), number of measurements n=4-16. The experiments on PET/Co and PET/Co +Fb (40 min) were run in duplicate.

Polyethylene terephthalate (PET), collagen type I impregnation (Co), laminin (LM), fibrin gel (Fb), fibronectin (FN), standard deviation (SD), non-significant (n.s.).

Fig. 4. Morphology of adhering human saphenous vein endothelial cells on PET vascular prostheses 4 h after seeding 1.50 × 10^5 cells/cm² (A-D) and 72 h after seeding (E-H). Staining with the Live/Dead Viability/Cytotoxicity Kit (Molecular probes), viable cells are in green. Olympus IX50 fluorescence microscope, Olympus DP70 digital camera, magnification 10 ×, scale bar 200 µm. Polyethylene terephthalate (PET), collagen type I impregnation (Co), laminin (LM), fibrin gel (Fb), fibronectin (FN).

Fig. 5. Morphology of human saphenous vein endothelial cells on PET/Co (A and D), PET/Co +LM (B and E) and PET/Co +Fb (C and F) vascular prostheses 72 h after seeding in static conditions (A-C) and after subsequent application of 120 min of flow (D-F). The flow direction is indicated by the arrow (D-F). Immunofluorescence of β-actin (green) (A and C, D-F) and of von Willebrand factor (green) (B) with cell nuclei counterstained with propidium iodide (red) (A-F). Leica TCS SP2 AOBS confocal microscope, magnification 10 ×, zoom 2 ×, scale bar 300 µm (A and D), magnification 10 ×, scale bar 200 µm (B and C, E and F). Polyethylene terephthalate (PET), collagen type I impregnation (Co), laminin (LM), fibrin gel (Fb).
The morphology of the HSVEC is presented in Figure 4. Viable cells are green, dead cells would be visible in red. However, there are no dead cells on any of the grafts. The adhering cells are becoming spread on PET/Co and PET/Co +LM (Fig. 4A,B); however, they are slightly less numerous and still round in shape on PET/Co +Fb and PET/Co +Fb/FN (Fig. 4C,D). After 72 h of growth – before they are submitted to the shear stress – the EC form a confluent monolayer, which seems to be more compact in the valleys (Fig. 4F,H) than on the crests (Fig. 4E,G) of the cramped (i.e. waved structure) prosthesis.

The population densities of the HSVEC after exposure to the flow conditions in a bioreactor are given in Figure 3B. They are compared in relative numbers to the static control prosthesis (= 100 %). The cell densities on PET/Co dropped to 78 % (n.s.) after 40 min of shear stress and to 61 % (p<0.001) after 120 min of shear stress, suggesting significant cell loss under flow of 2 h. The significant decrease in cell numbers was even more evident on the PET/Co +LM prostheses (55 %, p<0.001 and 50 %, p<0.001). However, no cell denudation was noted on the PET/Co +Fb vascular prostheses after both 40 min and 120 min of flow. Similarly, no cell loss was observed on PET/Co +Fb/FN during 120 min of shear stress application, though a significant decrease in cell density was noted after 40 min (68 %, p<0.05).

The morphology of the HSVEC on PET/Co, PET/Co +LM and in PET/Co +Fb in static conditions and after subsequent application of flow in dynamic conditions, as seen by confocal microscopy, is shown in Figure 5. The EC form a confluent monolayer after 72 h of static growth (Fig. 5A-C) and they become sub-confluent due to certain cell denudation after delivery of 120-min-shear stress on PET/Co and on PET/Co +LM (Fig. 5D and E). However, the EC maintained their endothelial phenotype, as shown by immunofluorescence of the cytoskeletal β-actin (Fig. 5A and C,D-F) and of von Willebrand factor, which is stored in the secretion granulae (Weibel-Palade bodies) in the cytoplasm of EC (Fig. 5B). Cell detachment on the cramped prosthesis occurred mainly in the regions of the crests (middle third of Fig. 5D and the upper and lower third of Fig. 5E), whereas the cells in the valleys remained attached (upper and lower third of Fig. 5D and middle third of Fig. 5E). No flow-dependent cell loss was observed when EC were seeded on PET/Co +Fb (Fig. 5F). In our study, we did not observe cell alignment with the direction of flow, since this physiological phenomenon occurs after longer application of shear stress, usually after 6-24 h (Punchard et al. 2007).

Discussion

Endothelial cell seeding on vascular implants was introduced in the 1980s, and it was proved to reduce the thrombogenicity of implanted prosthetic grafts (Ortenwall et al. 1990) and to enhance their patency (Meinhart et al. 2001). Impregnation of textile vascular prostheses with a collagen sealant was proposed in 1960 in order to make the graft impermeable to blood at the time of implantation; however, impregnated prostheses are not optimal substrata for direct endothelialization (Chakfé et al. 1999). To establish a cell monolayer on graft surfaces, optimal attachment and spreading of the cells is desired, together with the ability to withstand shear stress. Several research groups therefore investigated pre-coating vascular prostheses with cell-adhesive substrates and exposing the seeded cells to flow conditions (Rémy-Zolghadri et al. 2004, Bacakova et al. 2011).

EC seeding of rough-surfaced vascular prostheses with a tissue culture technique is problematic. Many authors have demonstrated the importance of providing a protein matrix to facilitate the adhesion, growth and retention of EC on prosthetic graft surfaces. Sentissi et al. (1986) cultured bovine aortic EC on ePTFE grafts pretreated with type I collagen and fibronectin. Cell detachment amounted to 7-11 % after 1-hour of flow (Sentissi et al. 1986).

Gourevitch et al. (1988) seeded HSVEC and human omental EC on PET grafts treated with various protein coatings. The 100-min adhesion was as high as 86 % on human cold insoluble globulin and 82 % on 1 % gelatin-coated grafts, and as low as 58 % on alginate-treated PET and 48 % on untreated PET. Compared to the initial cell density, they also reported EC retention of 73 % after 102 min of flow in an in vitro circuit on 6 mm gelatin-coated Dacron tubular grafts when perfused with a culture medium, and 64 % when perfused with heparinized human blood. This cell retention, however, was achieved after 30 min of maturation only (Gourevitch et al. 1988). Although the seeding efficacy in our study was much lower (Fig. 3A), the flow-dependent retention (40 and 120 min) of EC on our commercial PET/Co was comparable (78 % after 40 min and 61 % after 120 min) with gelatin-coated Dacron grafts.

The advantage of providing a protein adhesive
substrate to facilitate the endothelialization of vascular grafts was also demonstrated in vivo. Coating ePTFE grafts with fibronectin (FN) improved cell in-growth and surface endothelialization in a porcine interposition graft model; however, additional seeding of the FN-coated grafts with autologous EC (harvested from jugular vein) resulted in the most rapid EC coverage and excellent graft patency (Seeger and Klingman 1988).

Vohra et al. (1992) coated FN on ePTFE and gelatin on Dacron grafts. After seeding HSVEC (1.40 × 10^5/cm^2) on both coated samples they reached 60% seeding efficiency on ePTFE and 75% seeding efficiency on Dacron, at 90 min after seeding (Vohra et al. 1992). The cell retention after 120 min of shear stress (1.7 and 2.6 dyn/cm^2) was 55% and 57% on FN-coated ePTFE and 69% and 67% on gelatin-impregnated Dacron grafts, respectively. In our study, the seeding density was similar (1.50 × 10^5 HSVEC/cm^2), but the seeding efficiency amounted to 22% only on PET/Co after 4 h of culture. Under 40-min flow and 120-min flow, the resulting cell retention was again comparable – 78% on FN-coated ePTFE grafts and 61% on gelatin-impregnated Dacron grafts.

Pronk et al. (1994) investigated human peritoneal mesothelial cells (HMC) as an alternative source to EC for seeding Dacron vascular grafts. They found that the behavior of HMC was similar to that of human umbilical vein EC (HUVEC), and they reported 35% seeding efficiency after 60 min (2.50 × 10^5 HMC/cm^2) on plain Dacron and about 10% more on FN-coated (10 μg/ml) Dacron. Interestingly, a positive effect of FN pre-coating on cell adherence to the prosthesis was evident when the cells were seeded both in a serum-free medium (p=0.066, n.s.) and in a serum-containing medium (p=0.046, s.) (10% FCS) (Pronk et al. 1994). By analogy with this, we found 22% seeding efficiency of HSVEC on plain PET/Co, and this was further increased by surface pre-coating with LM up to 30% and with Fb/FN up to 27%. The slight discrepancy can be explained by the different cell type and by the lower seeding density in our study (1.50 × 10^5 HSVEC/cm^2). Similarly, we also seeded cells in a serum-containing medium (20% FCS).

However, Tunstall et al. (1995) found no enhanced adhesion of HUVEC (5.0 × 10^5/0.3 cm^2) to Dacron prosthetic material pre-coated with 1% gelatin, with FN (15 μg/ml) or with LM (6 μg/ml) compared to bare Dacron pre-incubated with the complete (i.e. 10% human serum-containing) medium. The authors reported the adherence of 50-70% of cells 2 h after seeding on 5 types of complete medium-pre-incubated Dacron grafts with different porosity (Tunstall et al. 1995). In our study, the variations found in cell adhesion to different substrata on PET/Co were statistically significant (Fig. 3A), and we also revealed differences by further testing the cell retention under flow conditions (Fig. 3B).

Sreerekha and Krishnan (2006) cultured ovine blood endothelial progenitor cells (EPC, 5.0 × 10^4/cm^2) on Dacron graft patches pre-coated with fibrin composite matrix (Fb, FN, gelatin, factor XIII and vascular endothelial growth factor – VEGF). They reported 2-hour adherence of 60%, 70% and 80% on bare Dacron, low-density Fb (2 mg/ml) and high-density Fb (10 mg/ml)-coated Dacron, respectively. Moreover, Fb induced differentiation of EPC into EC, as shown by staining of von-Willebrand factor, uptake of acetylated low-density lipoproteins (LDL) and nitric oxide (NO) synthesis. The cell proliferation over a period of 72 h and the NO production was also highest on high-density-Fb Dacron when compared to Fb-coated ePTFE. Applying shear stress (20 dyn/cm^2) for 1 h resulted in no cell loss, and the NO production under dynamic conditions was again highest on high-density Fb-coated Dacron (Sreerekha and Krishnan 2006). In our study, the 4-hour adherence of HSVEC was only 22% on PET/Co, 19% on PET/Co+Fb, and 27% on PET/Co+Fb/FN. This discrepancy may be due to the different prosthesis manufacturer and the use of a different cell type. Nevertheless, no cell loss during 2-hour flow on PET/Co+Fb and PET/Co+Fb/FN in our experiment with tubular grafts is consistent with Sreerekha and Krishnan’s study, confirming the concept that attached cells withstand the flow forces better on composite matrices resembling the natural ECM than on mono-protein layers.

The interaction of HUVEC with patches of commercial woven PET vascular prosthesis was extensively studied by Feugier et al. (2005). The cell adherence on plain grafts after 6-day culture was only 35% of the seeding density (1.05 × 10^5/cm^2); however, the adhesion increased to 83% when the graft surface was experimentally bio-functionalized with a type I/III collagen coating. The cell retention under the action of 60-min shear stress of 10 and 20 dyn/cm^2, in turn, amounted to 66% and 49%, respectively. Although they reported enhanced cell spreading under flow, the cell loss was still considerable on all of the grafts (Feugier et al. 2005). We studied HSVEC (1.50 × 10^5/cm^2) on knitted PET with commercial type I collagen impregnation, and
this data is in full accordance with our results – 78 % of HSVEC retained after 40-min SS, and 61 % retained after 120-min SS (12 dyn/cm²). Interestingly, the number of cells in the study by Feugier et al. (2005) that remained adherent after shear stress was significantly higher on the smoother surface of ePTFE grafts than on the more irregularly shaped surface of the woven PET. The authors explain that EC adhered to the PET fibers in a more elongated fashion than to ePTFE, thus forming sub-confluence, and that this may have caused the difference. Moreover, local irregularities of PET may give rise to local variations of shear stress and flow disturbances.

Wong et al. (2006) seeded ovine carotid artery EC (4.00 × 10^5 /cm²) on commercial knitted PET graft patches, and achieved coverage of about 30 % after 3-day culture and 56 % after 7-day culture. Cell retention under flow conditions decreased with increasing shearing and time of exposure values – 67 % after SS=0.018 N/m² (15 min) and 45 % after SS=0.037 N/m² (60 min) (Wong et al. 2006). In this experiment, both cell colonization in a static culture and cell resistance to flow were significantly better on PET grafts than on ePTFE grafts, in contrast to the findings of Feugier et al. (2005). Again, these results are comparable to our study with HSVEC on commercial knitted PET grafts.

Bérard et al. (2009) seeded human umbilical cord blood progenitor-derived EC (PDEC) on a commercial knitted PET prosthesis impregnated with bovine type I and III collagen in serum-free conditions. They found cell adherence of 20 % at 1 h, 21 % at 3 h, and 29 % (rounded values) at 24 h after seeding 2.00 × 10^5 PDEC/cm² on planar graft patches. Although the cells did not proliferate significantly until day 9 in static conditions, the application of flow for 6 h (after 3 static days) restored the endothelial monolayer in tubular grafts (Bérard et al. 2009). In our study, we seeded 1.50 × 10^5 HSVEC/cm² with very similar cell adhesion of 22 % after 4 h. The flow experiment with tubular grafts was performed in the same bioreactor (Fig. 2.) with very similar dynamic conditions (6 mm prosthesis, 15 dyn/cm²). By contrast, we observed significant cell denudation on the bare commercial PET/Co after 120 min of flow delivery. However, no cell loss was seen at 120 min when the cells were plated on PET/Co pretreated with either Fb or Fb/FN.

In our experiments, we chose crimped polyester vascular grafts (i.e. a wavy structure). These grafts have the clinical advantage of possible elongation (e.g. when the graft runs over a joint) and better resistance to bending (Hajjaji et al. 2012). The direction of the blood flow is perpendicular to the direction of crimping (Fig. 5D-F), and it runs over crests and valleys about 1 mm in length (Fig. 1A). Crimped vascular grafts are usually implanted with a certain degree of tension in an elongated fashion between the two sites of anastomoses to the native artery, thus partially removing the effect of crimping. However, in our study, the investigated prosthesis was mounted to a connector on one side only, thus retaining the crimped structure. The flow pattern had previously been investigated in a model of the crimped vascular grafts used in our study. Although a certain backflow was observed in the crimped tubes, no significant impact of crimping was found on the laminar flow pattern (Konfršt et al. 1994, 2005, Abdessalem et al. 1999). Moreover, no clinical difference between crimped and non-crimped grafts was found in terms of autologous cell seeding in a dog model (Baitella-Eberle et al. 1991). Interestingly, the HSVEC in our in vitro study were shed during flow more on the crests than in the valleys of the crimped grafts; however, this happened mainly on PET/Co and PET/Co +LM (Fig. 5D and E) – these are the grafts that sustained major cell detachment during flow.

To summarize current knowledge, it is evident that human EC of various origins can colonize commercial PET vascular grafts in vitro. However, substantial cellular detachment still occurs even after short-time action of flow conditions that represent the clinical situation. The contribution of our experiment consists in confirming these observations in clinically-used tubular 3D bypass grafts submitted to flow in a hemodynamic chamber.

Laminin coating has previously been shown to increase both cell attachment and cell retention, but no difference from other ECM molecules was demonstrated (Salacinski et al. 2001). Surface modification using LM was reported to accelerate endothelialization of porous vascular grafts in rats (Williams et al. 2011). However, all these tests were carried out on PTFE. The seeding efficiency of HSVEC on PET/Co +LM in our study was the highest – 30 % of seeding density; however, the cell numbers significantly dropped to 55 % after 40 min of flow, and to 50 % after 120 min of flow, compared to static conditions.

When the PET/Co prostheses in our study were coated with Fb or Fb/FN assemblies, there was much better cell retention during the flow experiment, and no significant cell denudation was noted under shear stress up to 2 h (Fig. 5F). The EC adhere to collagen through
integrin receptors $\alpha_1 \beta_1$ and $\alpha_2 \beta_1$, and to laminin through $\alpha_2 \beta_1$, $\alpha_5 \beta_1$ and $\alpha_6 \beta_1$. Binding of fibronectin requires $\alpha 3 \beta 1$, $\alpha 4 \beta 3$, and $\alpha v \beta 3$, and binding of fibrin also requires $\alpha 4 \beta 1$ and $\alpha 5 \beta 1$ (Hynes et al. 2007, Takada et al. 2007). Integrin receptors $\alpha v \beta 3$ and $\alpha 5 \beta 1$ play a crucial role in the adaptation of EC to hemodynamic force in terms of integrin activation, intracellular signaling and change in adaptation of EC to hemodynamic force in terms of specific signals (mediated by integrins $\beta 1$, $\beta 3$ and $\beta 5$). In this study, it was observed that fibrin or fibrin/fibronectin assemblies can provide the seeded EC with more matrix-specific signals (mediated by integrins $\beta 1$ and $\beta 3$) that elicit desired cellular responses under shear stress.

The advantages of using fibrin as a substrate for cell seeding are that it can be isolated from autologous blood, it contains recognition sites for growth factors and cell adhesion receptors, and it induces matrix synthesis and differentiation of EC (Filová et al. 2009, Riedel et al. 2009). Moreover, a combination of fibrin gel with additional ECM components (e.g. FN) preserves EC adhesion and proliferation, and enables the formation of more mature contacts of focal adhesion (Filová et al. 2014). This was demonstrated by the higher cell density on PET/Co +Fb/FN than on PET/Co +Fb (Fig. 3A).

Kjaergard and Weis-Fogh (1994) described a technique for sealing a high-porosity polyester prosthesis with autologous fibrin obtained from patients’ own blood. The grafts could withstand pressure of 300 mm Hg, possessed good handling characteristics, and had no risk of any disease transmission (Kjaergard and Weis-Fogh 1994). Similarly, the commercial sealant of knitted PET vascular prostheses was experimentally replaced by autologous fibrin matrix, and these grafts were seeded with bone marrow cells in a porcine model. These constructs showed lower risk of short-term (2 weeks) neo-intimal formation and thrombosis than grafts pre-clotted with whole blood; however, 3-month implantation resulted in substantial intimal hyperplasia, possibly due to the multi-potency of bone marrow cells (Cardon et al. 2000). Hasegawa et al. (2007) implanted 2 mm thrombin-free fibrin-coated knitted polyester vascular grafts in carotid arteries in a rabbit model. Reduced platelet deposition and infiltration of immune cells, decreased expression of inflammatory markers, and improved fibrinolytic balance were observed when autologous fibrin was used rather than when fibrin of xenologous origin was used. In addition, spontaneous endothelialization was promoted (Hasegawa et al. 2007).

Fibrin glue has previously been used as a support for endothelialization in arterial prosthetic grafts, both experimentally (Fernandez et al. 2007) and in human trials, and it has been clearly shown that these conduits have better clinical performance (Meinhart et al. 2001, Deutsch et al. 2009); however, the underlying bypass graft material was ePTFE in most cases. A further exceptional contribution of our study is that we have adopted the approach of coating fibrin gel or fibrin/fibronectin assemblies onto clinically-used PET/Co bypass grafts. These types of vascular prostheses have been clinically implanted for decades; however, the lack of an endothelial lining in human often results in thrombotic events with serious health complications. Our results are clinically relevant with potential direct clinical application, because we used endothelial cells derived from patients with cardiovascular disease, and our experimental coating of the prosthesis resulted in excellent shear stress resistance of the seeded cells.

Conclusion

Human primary endothelial cells can sufficiently colonize a bare commercial knitted PET/Co vascular prosthesis in a serum-containing medium; however, significant cell detachment occurs after flow is applied. Coating the inner surface with laminin improves the cellular adhesion, but the shear stress resistance remains poor. Conversely, the resistance of the adhering cells to dynamic flow conditions mimicking the blood flow is significantly enhanced when the cells are plated on prostheses modified by a fibrin network or by fibrin/fibronectin assemblies. These types of modifications may be suitable for the purposes of tissue engineering of vascular grafts.

Conflict of Interest

There is no conflict of interest.

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