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Adhesion Molecule-Modified Cardiovascular Prostheses: Characterization of Cellular Adhesion in a Cell Culture Model and by Cellular Force Spectroscopy

U. Bakowsky, C. Ehrhardt, C. Loehbach, P. Li, C. Kneuer, D. Jahn, D. Hoekstra, and C.-M. Lehr

Abstract

In vascular surgery the insertion of artificial blood vessels is a common method, but limited to specific medical applications. Long-term studies have revealed that unspecific adhesion of fibrin and collagen to the implant surface leads to thrombus formation and occlusion, even though PTFE is extremely inert and hydrophobic.

In the present study we describe the modification of cardiovascular prostheses made of PTFE by a chemical method in combination with a biochemical method, leading to improved cell adhesion. The surface was covalently coated with extracellular matrix-adhesion molecules and with a fragment of the bacterial invasin protein of *Yersinia enterocolitica*, the so-called Min3. All these molecules showed high binding constants for $\alpha 4$–$\beta 1$-integrins, which were expressed on the surface of endothelial or fibroblast cells. On this protein-modified surface, primary endothelial cells (HUVECs) were seeded in the presence of growth factors, and cells were exposed to physiological shear stress after an initial adhesion period. To characterize the adhesion properties of the HUVECs, we used scanning force microscopy (SFM). In this study, we developed a method to determine the specific interactions of HUVECs with various adhesion molecules using SFM. HUVECs were grown at the edge of a "tipless" cantilever. We observed higher cell adhesion rates and higher adhesion forces when using Min3 than for collagen or fibronectin. The measured adhesion force depended on the cell surface area and the contact time (between the implant and the cantilever), and ranged between 0.2 and 1.2 nN.

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11.1 Introduction

Cardiovascular disease is the primary cause of death, in both the United States and Europe, with arteriosclerosis being the most common form. The process of arteriosclerosis is considered to consist largely of the accumulation of lipids within the artery wall [1]. By surgical therapy new pathways (conduits) are being constructed, connecting the aorta or other major arteries and distal segments of arteries (e.g., coronary arteries) with vessels beyond the stenosis or obstructing lesions. By this means, blood supply to ischemic tissues can be re-established [2]. Reversed segments of autologous saphenous vein can be used as appropriate conduits. Particularly in coronary revascularization, the internal mammary arteries and the radial arteries have widely been used [3]. However, autologous vessels may be insufficient for multiple or repeated bypasses and/or saphenous veins may have varicose degenerative alterations. Therefore, allograft arteries and veins as well as synthetic tubes have been developed, but they proved to be less satisfactory as conduits [4, 5]; particularly, synthetic grafts with an internal diameter of 6 mm or less are prone to thrombus induction and occlusion [6].

In this context, the development of novel biocompatible materials becomes more and more important. Current grafts are restricted to large and medium diameters since none of the available ones is suited for the replacement of small-diameter vessels or coronary arteries, respectively.

A slight blood flow favors the formation of thrombi, resulting in a subsequent occlusion of the vessel. By administration of anticoagulative drugs, this outcome can be reduced, but over the years the formation of a neointima occurs, which consists of both stromal cells and endothelial cells. In particular, stromal cells are prone to hyperplasia, thus steadily reducing the vessel lumen, which results eventually in the formation of a stenosis or the occlusion of the prosthetic graft.

Local clotting seems to be the central mechanism of thrombus formation in arteriosclerosis of native blood vessels and limits the performance of prosthetic grafts [7–11]. The principle of *in-vitro* endothelialization of vascular grafts has been established and clinical results are available [12–17]. In this case, a lining of vascular grafts with endothelial cells is possible, but a confluence exceeding 70% of the inner surface, can only be reached by the use of growth factors in the culture medium. Apparently, this limitation in cell density is the reason for the fact that present applications with endotheliazed grafts show no significant refinement [14, 18–23]. One explanation for these difficulties is the small cell yield obtained from isolation of donor vessels; it can be increased by addition of growth factors, but then cells might undergo dedifferentiation in batch culture. Another problem occurring with dedifferentiated cells is the adherence of leukocytes to their surface, which in turn leads to the formation of a thrombosis, resulting – in the worst case – in an occlusion of the vessel. In addition to that, insufficient adherence of endothelial cells to the vascular graft, caused by the inertness and hydrophobicity of the polymer wall material, leads to more than 80% of the cells lining the graft being washed away when physiological shear stress is applied. Two biomaterials dominate the vascular

graft market to date, Dacron (PET, poly(ethylene terephthalate)) and PTFE (polytetrafluoroethylene). Dacron monofilaments are woven or knitted into various designs to form the graft. They are generally used in the large-diameter category (12–22 mm). Expanded PTFE consists of nodes and fibers and was generally used in the intermediate-diameter category (6–12 mm). The porosity of these PTFE grafts encourages the formation of a biological lining on the luminal surface, known as neointima (cellular lining) or pseudointima (acellular lining). Therefore, modified PTFE grafts are more suitable candidates for small-vascular prostheses than PET. The concept of endothelial cell seeding on vascular implant surfaces was developed to mimic physiological blood vessels and to further reduce unspecific adhesion [11, 24]. PTFE, however, is a very hydrophobic matrix and thus not the optimum substrate cell culture. Adhesive coatings with aqueous proteins on the implant surface have given only inadequate performance under shear stress conditions [25–27]. Therefore, covalent attachment of specific adhesion molecules may provide a means to achieve a strong, shear stress resistant, cell binding.

In this study, we describe the modification of PTFE by a chemical method in combination with a biochemical method leading to an improved cell adhesion. The modification was first developed with punched disks from PTFE film, and was then transferred to commercially available vascular grafts made of PTFE. The aim was to retain the mechanical properties of the polymer so that only the biological properties of the inner surface of the graft were changed. Thus it would become possible to couple adhesion proteins to the previously inert and hydrophobic polymer surface to favor adhesion and attachment of endothelial cells (ECs) withstanding shear stress as it occurs in small-diameter vascular grafts.

We tested a three-step-preparation in order to alter the polymer surface selectively. In the first of these steps, reactive groups should be formed on the inner surface of the hydrophobic PTFE, which than make it possible to couple a crosslinker for further attachment of the adhesion molecules. Reactive surface groups such as hydroxyl, amino, or carbonate can be generated on formerly inert PTFE surfaces by different methods, including dry-chemical treatment (e.g., plasma etching, ablation) or wet-chemical methods (e.g., treatment with H_2O_2/H_2SO_4, chromate, permanganate) [28–31]. The activation of PTFE tubes with H_2O_2/H_2SO_4 as oxidant was our first choice, because it resulted in a sufficient number of free functional groups without damaging the structural integrity of the polymer combined with good handling. In a second step, the crosslinker, cyanuric acid, was covalently bound via hydroxyl groups to the activated PTFE. Cyanuric acid theoretically provides two binding sites, hydroxyl and amino groups, for proteins. The family of extracellular matrix (ECM)-proteins (e.g., laminin, collagen, fibronectin) and their derivates (RGD peptide sequences) came into consideration for the final functionalization of the activated and crosslinked polymer. Here, we struck out on a new path by choosing a fragment of the bacterial invasin protein of *Yersinia enterocolitica*. This bacterium enters mammalian organisms via the gut and the protein that enables this access is called invasin A.

Primary human umbilical venous epithelial cells (HUVECs) were seeded on this protein-modified surface. Culture was performed in the presence of growth

factors and cells were exposed to physiological shear stress after an initial adhesion period.

To characterize the adhesion properties of the HUVEC, we used scanning force microscopy (SFM). In this study, we developed a method to determine the specific interactions of human endothelial cells (ECs) with various adhesion molecules using SFM. The ECs were grown at the edge of a tipless cantilever.

The adhesion molecules (i.e., gelatin, fibronectin, collagen, Min3) were covalently coupled to an artificial blood vessel made from PTFE. Due to unspecific adhesion of fibrin and collagen to the implant surfaces, thrombus formation and occlusion may occur in clinical use. To overcome this problem, the concept of EC cultivation on modified implant material was developed to mimic the physiological surface of blood vessels [36]. The expiration of these cell layers strictly depends on the cell-surface interaction, which can be improved by modifying the graft surface with adhesion molecules.

11.2 Materials and Methods

11.2.1 Chemicals for the Modification

H_2SO_4 and chloroform were obtained from Merck, H_2O_2 from Roth, and cyanuric acid from Sigma. All chemicals used for the modification were of analytical grade. Collagen, fibronectin, gelatin, and BSA (bovine serum albumin) were purchased from Becton Dickinson. The adhesion molecule from *Yersinia enterocolitica*, Min3, was expressed in *Escherichia coli*, isolated, and purified by D. Jahn's group (Universität Braunschweig).

11.2.2 Implant Materials

Commercially available PTFE was used for the investigations (Merck). We developed the method and the parameters (acids, concentration, ratio, time) for the wet-chemical modification of PTFE on the basis of PTFE film material (thickness 0.1 mm) punched in 12 mm disks. Then the results from the PTFE film model were transferred and adjusted to medical grade vascular grafts (B. Braun AG; see Fig. 11.1).

11.2.3 Modification of the PTFE Surface

In a first reaction step, the surface was treated with H_2O_2/H_2SO_4 (1:1) at room temperature for 20 min. In the second step, the reactive hydroxyl groups generated were esterified with cyanuric chloride with release of HCl. For activation,

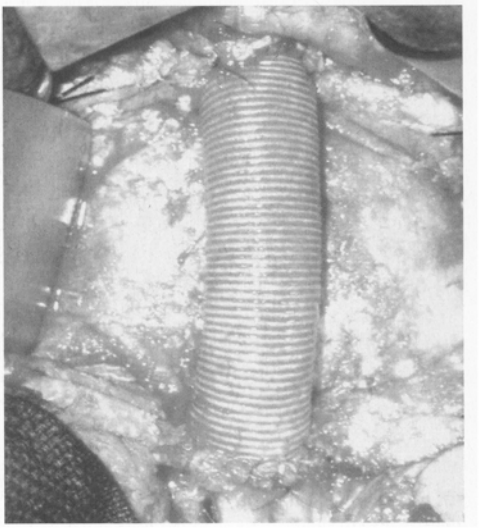


Fig. 11.1 Cardiovascular prosthesis used for surgical treatment of an aneurism of the aorta in a real patient. Courtesy of Prof. Dr. H.-J. Schaefers (Homburg/Saar, Germany).

we used a filtered 10^{-3} M solution of cyanuric chloride in chloroform for 1 h followed by intensive washing with chloroform to remove excessive cyanuric chloride (Figs. 11.2, 11.3). In the third step, the activated PTFE surface was finally coupled with functional molecules to achieve targeted adhesion and spreading of endothelial cells. High affinity and specific interactions of these molecules with physiological receptor molecules on the cells were of importance. Candidate adhesion molecules are constituents of the extracellular membrane (collagen, fibronectin, lami-

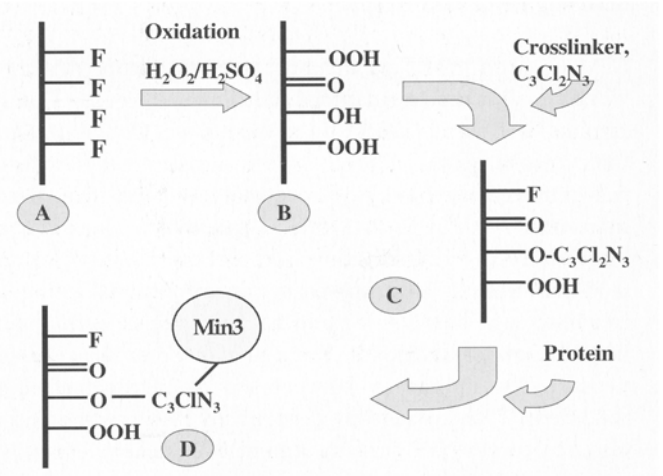


Fig. 11.2 Reaction scheme of the wet-chemical modification of PTFE. (A) unmodified PTFE; (B) oxidized PTFE with possible reactive groups formed; (C) binding of the crosslinker cyanuric chloride; (D) covalent binding of adhesion protein to PTFE.

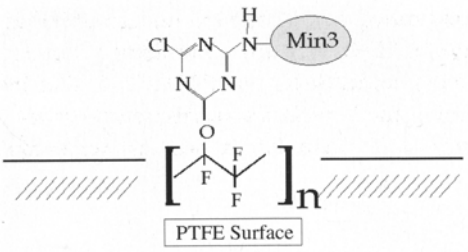


Fig. 11.3 Schematic of PTFE modified with Min3.

nin, vitronectin), other peptides and proteins containing the RGD motif, or adhesion proteins of microorganisms such as the invasin A of *Yersinia* species, and derivational sequences. These adhesion proteins target specific receptors on the cells, especially $\beta 1$ and $\beta 3$ integrins. In earlier experiments, we observed a higher cell adhesion rate during 24 h of incubation when using the bacterial invasin (Min3) than with collagen or fibronectin. This molecule binds specifically and with a high affinity to $\alpha 4$–$\beta 1$ integrins (vla-4) [15]. The association constant is approximately three magnitudes higher than for the commonly used extracellular matrix proteins (collagen, fibronectin) and RGD-peptides. Min3 was produced by heterologous expression in *E. coli* and purified by maltose-binding affinity chromatography and ion-exchange chromatography to a purity of >95%. After intensive washing with chloroform, an aqueous solution of the test adhesion protein (borate-buffer, pH 8.4) was incubated for 12 h to allow reaction of surface-bound cyanuric chloride residues with amino groups of the protein.

11.2.4
Scanning Force Microscopy

SFM was performed on a Nanoscope IV Bioscope (Veeco). The microscope was vibration- and acoustic-damped (Brunhild II, Veeco). Commercially available pyramidal Si_3N_4 tips (Veeco) on a V-cantilever (length 125 µm) were used for the force measurements. For the force measurements with attached ECs, the tip was removed mechanically from the cantilever. The measurements were done in contact mode with PBS (pH 7.4) medium. The force constants of the cantilevers were determined according to Florin et al. [37] and were in the range 0.06–0.14 N m^{-1}. The scan speed was proportional to the scan size and the scan frequency was between 0.5 and 1.5 Hz. Images were obtained by displaying the height signal and friction force signal, both signals being recorded simultaneously. The interaction between the cantilever-attached ECs and the protein-modified PTFE surface was determined by recording and analysis of the force–distance curves. For the attachment of ECs to the nanosensor, the cantilevers were pre-activated with cyanuric chloride and modified with Min3. The cells were cultured on the cantilever for three days post-seeding, used for the experiment, and afterwards analyzed by scanning electron microscopy (SEM). Cells were successfully attached on three out of ten cantilevers.

The visualization of the protein modification was performed under normal atmospheric conditions, at a temperature of 25 °C and with a humidity of approx. 60%. We used commercially available pyramidal Si_3N_4 tips (NCH-W, Digital Instruments, Santa Barbara, CA) in tapping mode with a scan frequency between 0.5 and 1.5 Hz, a resonance frequency of 220 kHz, and a nominal force constant of 36 N m^{-1}.

11.2.5
Fourier Transform Infrared Spectroscopy

The surface modification was further followed by FTIR (ATR) spectroscopy on a Perkin-Elmer System 2000. Analysis was performed with an internal reflection element (GaAs crystal, 45° incidence angle) with 100 scans co-addition and a resolution of 4 cm^{-1}. The insoluble, inert PTFE was cut into thin microtome slices between 150–500 nm and analyzed in transmission mode.

11.2.6
Environmental Scanning Electron Microscopy

The ESEM (XL30 ESEM-FEG, Philips) that was used warranted a lateral resolution of 3.6 nm and was therefore qualified to visualize the global morphological appearance of the polymer surface and to characterize the cell-modified nanosensors (cantilevers).

The unmodified and chemically activated specimens were sputtered with platinum without any other treatments. The cell-covered specimen (cantilevers and PTFE) were fixed with 2.5% glutaraldehyde–phosphate-buffered saline solution (Sörensen/Arnold; pH 7.4) for 2 h and, after extensive washing, drained in an ascending ethanol column (30, 50, 70, 80, 90, 96, and 100%). After this procedure, the specimens were transported in water-free acetone to remove alcohol, subjected to critical point drying, and sputtered with platinum. All specimens were investigated under vacuum conditions (10^{-3}–10^{-4} atm).

11.2.7
Confocal Laser Scanning Microscopy (CLSM)

For CLSM, PTFE grafts lined with endothelial cells were treated as follows. Specimens were fixed in 4% paraformaldehyde (PBS, pH 7.4) for 15 min, washed with PBS, and permeabilized with a 2% Triton X-100 solution in PBS for another 15 min. After washing again with PBS, they were incubated with the primary monoclonal antibody vWF (CD31) (DAKO) in a 1:50 dilution at 37 °C for 30 min. After another washing step, the specimens were incubated with the secondary antibody (FITC-labeled, DAKO) in a 1:100 dilution at 37 °C for 30 min. Then, cell nuclei were stained with a 1:1000 solution of propidium iodide at 37 °C for 15 min. After extensive washing and a short air drying, the PTFE matrices were mounted onto glass slides with FluoroSave (Calbiochem)

and hardened overnight. The specimens were studied with an MRC1024 confocal laser scanning microscope (BioRad).

11.2.8
Isolation and Culture of HUVECs

HUVECs were isolated within 6 h of childbirth [38]. During transport and processing, umbilical cords were stored in sterile HBSS (Hanks' balanced salt solution) supplemented with an antibiotic–antimycotic solution (Sigma). Ensuring sterility, 1 cm lengths of both ends of the umbilical cord were cut off and the cord was cannulated immediately from both ends with a nub canula (Medica) and kept in place with mosquito clamps (Medica). Subsequently, the vein was perfused with sterile HBSS until the effluent was free of red blood cells. After the vein had been depleted of air, it was filled with 0.25% collagenase type I solution (Worthington) prewarmed to 37 °C and plugged with Luer lock plugs. The cord was then incubated for 15 min at 37 °C. For increased cell yield, the cord was gently massaged and the vein was flushed with 50–100 mL of 10% FCS (Foetal Calf Serum) in HBSS. The collected effluent was centrifuged at 500 *g* for 10 min. The cell pellet was washed twice with HBSS and centrifuged again at 500 *g* for 10 min. The cells were resuspended in EGM II culture medium (Promocell), filtered through a piece of sterile gauze, and seeded into 75 cm^2 culture flasks (Greiner) freshly coated for 30 min with 0.1% gelatin in phosphate-buffered saline (PBS). Cell cultures were incubated at 37 °C in a 5% CO_2 atmosphere. The medium was changed every second day. When they were approaching 70–80% confluence, cells were harvested using trypsin/ethylenediaminetetraacetate (EDTA) (Sigma) and passed onto gelatin-coated culture flasks at a split ratio of 1:4.

11.2.9
Endothelialization of PTFE Films

Cell seeding experiments were performed after functionalization of the PTFE surface with various proteins to test the biological adhesiveness.

From the oxidized and crosslinker-coupled film, 12 mm disks were punched and mounted in Minusheet carriers (Minucell) which were placed in 24 microtiter plates. The adhesion protein solution was added and incubated overnight at 4 °C. After being washed with prewarmed PBS, the endothelial cells were left to adhere for 24 h on the PTFE surface under static conditions. For perfusion experiments, six Minusheet carriers were placed in a perfusion container (Minucell) and physiologic shear stress (about 200 s^{-1}) was applied for 72 h, to investigate the stability of the EC lining. The physiological blood viscosity (3.3 mPa s) was mimicked with a DMEM medium (10% FCS, HEPES, PenStrep) containing 6% dextran (70 000 Da).

11.3
Results and Discussion

11.3.1
Wet-Chemical Modification of PTFE Polymer Film

One strategy to reduce occlusion events in small-diameter PTFE grafts can be to line these grafts with endothelial cells. Therefore, a three-step procedure to selectively modify the PTFE polymer surface was developed.

Variation in incubation time and temperature changed the gain of reactive hydroxyl groups obtained after the treatment with H_2O_2/H_2SO_4. A cross-sectional analysis of the PTFE by SEM showed that the oxidation could be observed to occur in a depth up to 20 nm. Verification of free hydroxyl groups was performed by FTIR. Untreated PTFE showed characteristic peaks between 1000 and 1400 cm^{-1}. After oxidation, new peaks were found at 3300–3500 cm^{-1} and around 1650 cm^{-1} (indicative of OH groups). The progression of the oxidation reaction was also followed by measurement of the equilibrium contact angle with water. A significant decrease of the contact angle from 125° to 88–115° was noticed, depending on the reaction parameters. No visible changes of the surface morphology were observable by SEM and SFM.

While the surface morphology of PTFE remained unchanged after the second reaction step, the reaction could be followed and characterized by FTIR. The spectra showed additional peaks corresponding to C=N bonds at 2200–2400 cm^{-1} (2450 cm^{-1}). The contact angle with water increased to 110° after reaction with cyanuric chloride. The stability and elasticity of PTFE remained unaffected.

The multiple steps of the modification from oxidation to the attachment of the protein were observed using the contact angle method, but an exact quantification was difficult. The oxidation yielded enough reactive groups such as hydroxyls, ketones, aldehydes, as well as chain breakage. The diameter of the cyanuric chloride molecule is in the region of 1 nm, hydroxyl groups are 0.2 nm and a single protein covers 8–10 nm. For comparison, lipid head groups occupy approximately 0.4 nm^2. The hydroxyl group is much smaller and when the protein is covalently bound to the surface, approximately 200 molecules of cyanuric chloride would be covered under the assumption that the surface is homogenous and tightly packed. This indicates that only a low degree of surface activation is required for the intended modification.

Changes in surface morphology during modification were followed by SFM. No changes in the overall surface morphology were detectable after oxidation, activation and crosslinking with the protein. This indicates that this method has a good selectivity. Using the high resolution of the AFM technique, it was possible to visualize the attachment of single protein molecules. These appeared as round structures with a diameter of 8–10 nm. This size corresponds well with the molecular weight of Min3 (117 kDa). It was also possible to quantify the degree of modification: There were 408 ± 28 proteins per μm^2 on a total analyzed area of 100 μm^2 (Fig. 11.4 A). For BSA, collagen IV, and fibronectin a coupling

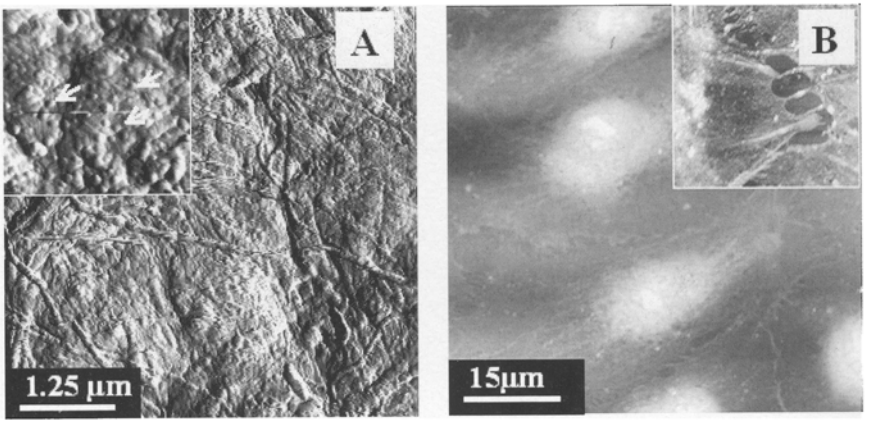


Fig. 11.4 AFM images of a Min3-modified PTFE surface (A) before and (B) after the growth of endothelial cells. (A), insert: globular structures with a diameter of 9 nm, comparable in size with the adhesion protein Min3; (B), insert: formation of tight junctions between the individual cells.

density between 95 molecules for collagen and 475 molecules for BSA could be observed. The surface density of biomolecules depends on the size of the molecule, the charge, and the adsorption behavior.

The surface modification could be followed further by FTIR spectroscopy. After functionalization, typical protein peaks were detected at 1639 (amide I), 1548, and 1122 cm^{-1} (amide II), and further peaks at 3415 and 3214 cm^{-1}.

11.3.2 Cell Adhesion Experiments

11.3.2.1 Adhesion and Cultivation in Static Culture

The PTFE graft, functionalized with various adhesion molecules, showed an almost confluent lining of the surface with ECs during static culture for all the protein coatings used. Differences could only be observed for the time when maximum confluence was reached (Fig. 11.4 B). Then, only the untreated material and the PTFE coated with BSA (an inactive control) showed an incomplete cell lining. The stability of the EC layers to washing with phosphate buffer increased with rising binding strength of the ligand, in the rank order: BSA < fibronectin < collagen < Min3.

11.3.2.2 Perfusion Experiments

Endothelial cells seeded on untreated PTFE or BSA-modified PTFE were totally washed away when shear stress was applied. As shown in Fig. 11.5, only the endothelial cells cultivated on Min3-modified PTFE could withstand the shear

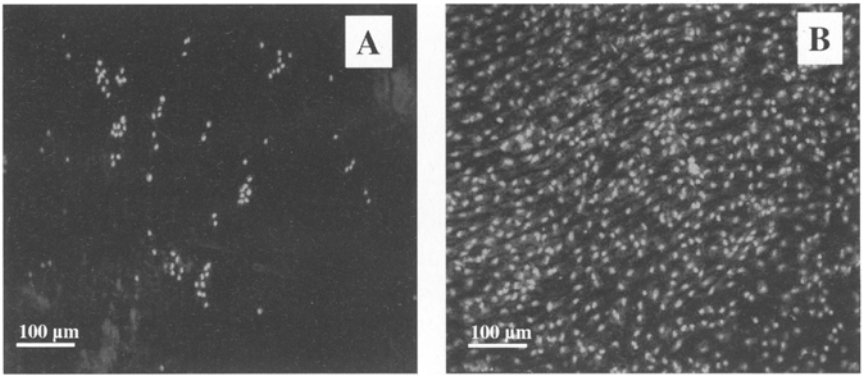


Fig. 11.5 Cell adhesion experiment. Confocal laser scanning microscopy of endothelial cells growing on (A) unmodified and (B) Min3-modified PTFE.

stress, and remained on the surface. Nearly 90% of the total implant surface was still covered with cells after 72 h of perfusion. The ECs were found to be aligned in the flow direction.

11.3.3 Cell Adhesion Force Measurements

In this study, the surface morphology of the implant material and the coupled adhesion molecules could be visualized at molecular resolution in the native wet state by SFM. By scanning the PTFE surface with the cell-modified cantilever, the adhesion properties of the ECs were assessed (Fig. 11.6). When an unmodified or BSA-coated surface was used, a clear image of the probe was achieved. The lateral resolution of the SFM was significantly decreased, because of the decrease in sharpness of the tip on the cantilever (i.e., the ECs were the sensor). When ECM adhesion protein molecules were attached to the surface, strong adhesion of the cantilever/cell nanosensor could be observed, which was evident by the horizontal streaks on the lateral force image (Fig. 11.7 A). This effect could be shown for all adhesion molecules (i.e., Min3, collagen and fibronectin). When the interaction was blocked by injection of an excess of soluble adhesion molecule, a clear image of the surface was regained (Fig. 11.7 B). In force scans (Fig. 11.8), the deflection of the cantilever was recorded on its approach to the PTFE surface and on retraction from the surface, and was directly converted into adhesion force. The calculated results are summarized in Fig. 11.9. Only low adhesion forces were observed by scanning the unmodified or BSA-coated surface (between 0 and 50 pN). When the surface was modified with Min3, fibronectin, or collagen, a drastic increase in the adhesion forces was observed (Fig. 11.9, upper graph). This increase was significantly higher when using Min3, than for fibronectin and collagen. The applied adhesion force

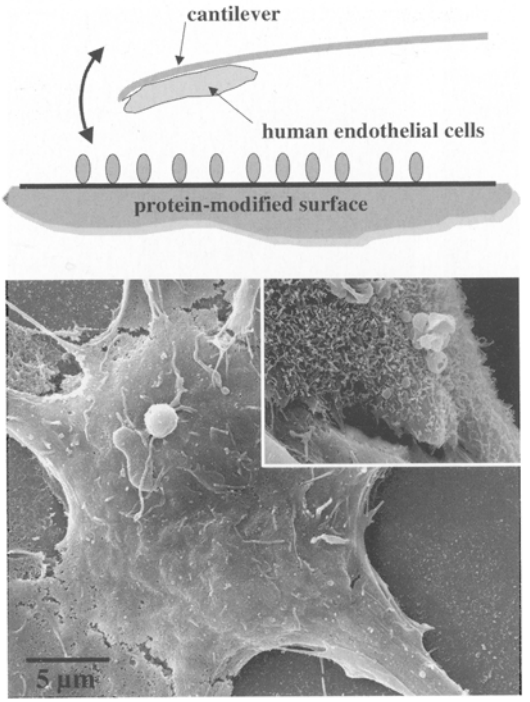


Fig. 11.6 Experimental set-up for the force measurements. The cantilever with the attached endothelial cells scans the protein-modified PTFE surface and endothelial cells grown on a silicon cantilever (EM image). Insert: 5 µm×7 µm.

Fig. 11.7 Scanning lateral force microscopy. (A) Min3-modified surface; the streaks indicate a strong adhesion during the visualization of the surface; (B) the same surface area was scanned, but the interaction was blocked by soluble Min3.

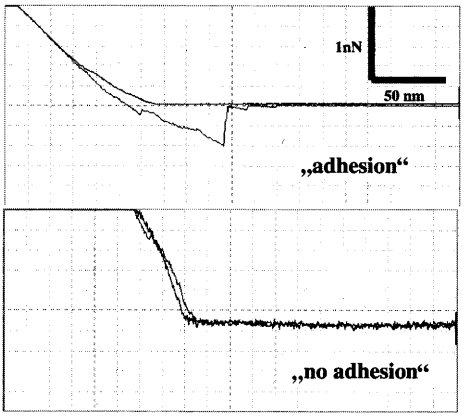


Fig. 11.8 Individual force vs. distance curves determined by measuring the adhesion of cell-modified cantilevers on modified implant materials. Adhesion time: 2 s.

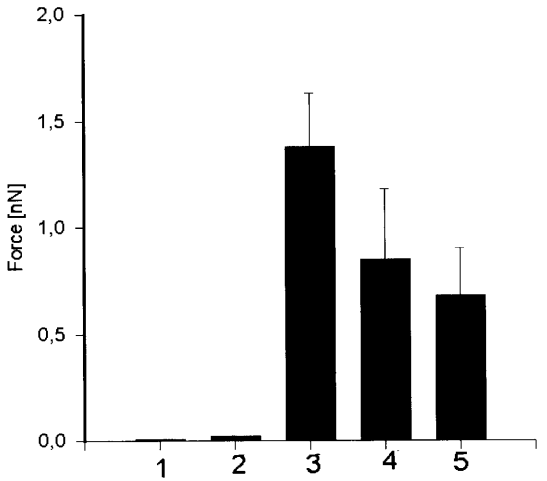
for all ECM proteins was found to be in the range of some nano-newtons. The total adhesion forces were dependent on the real surface area of the sensor-attached cell, which was in contact with the modified implant material. For each cantilever, varying forces could be observed. The results shown were calculated of the measured forces of at least six individual cantilevers.

It could be shown that the adhesion force also depended on the contact time between the cantilever and the implant surface. Longer adhesion times led to higher adhesion forces (Fig. 11.9, lower graph). This fact can be discussed as a time limitation on the bond formation between the individual ligand–receptor pairs and is in agreement with the published literature [38].

11.4 Conclusion

Cell-adsorptive coatings of PTFE with laminin, collagen IV, and fibronectin have been described in the literature to improve the growth of endothelial cells on vascular implants, but these methods did not show satisfactory results when applied to vessels with a small diameter.

Here, we report on the covalent attachment of a similar adhesion protein, Min3. The improved performance of this modification can be attributed both to its higher receptor affinity compared with ECM proteins and to the covalent fixation to the PTFE. The latter especially might be of importance, to resist shear stresses as encountered in the systemic circulation. In addition, in this study the force interaction between a human EC and a number of adhesion proteins was assessed for the first time. This was made possible by growing ECs on the tip of an SFM cantilever and scanning across a protein-coated PTFE graft surface. The strongest interaction was found when the bacterial invasin Min3 was used, followed by fibronectin and collagen.



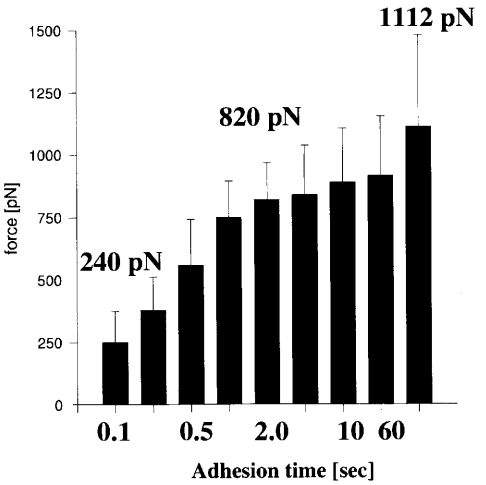


Fig. 11.9 Upper graph: Calculation of adhesion forces between the protein-modified PTFE surface and the cantilever-attached EC. 1: unmodified surface; 2: BSA; 3: Min3; 4: fibronectin; 5: collagen IV; adhesion time: 2 s. Lower graph: Dependence of calculated adhesion forces between the Min3-modified PTFE surface and the cantilever-attached EC on the contact time (between 0.1 and 60 s). The adhesion force increased from 240 pN (0.1 s contact) to 1112 pN (60 s contact).

Acknowledgments

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