Plasma Treatment of Vascular Implant

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Abstract. This paper examines a carbon layer on a blood vessel implant. The carbon layer is created by high energy ions bombarding an inner surface of the polyurethane implant. An analysis of the molecular structure of the layer was carried out, demonstrating the appearance of free radicals stabilized by aromatic clusters, as well as new carbon-carbon, oxygen-containing and nitrogen-containing groups in the inner surface of the blood vessel implant. The covalent adsorption of the protein on the carbon layer inside the implant has been proven. The antithrombogenicity of the carbon layer with a covalently bound protein on polyurethane implant of blood vessels in an experiment on rabbits has been shown.

1. Introduction
Cardiovascular diseases are the leading cause of death worldwide [1, 2, 3]. About 17 million people die from heart disease each year, accounting for approximately 29% of all deaths. The largest number of deaths from cardiovascular diseases occurs at the age of 45-50 years, that is, during the working period. Increasing the life expectancy of patients with cardiovascular diseases is often possible only with endoprosthetics of blood vessels and heart valves, installation of stents or pacemakers. However, when implanted into a patient's body, synthetic materials cause a foreign body reaction, which is accompanied by the formation of fibrous tissue and thrombotic masses around the endoprosthesis [4, 5]. Back in 1979, N. Haimow, F. Giron and JH Jacobson observed 362 Gore-Tex expanded polytetrafluoroethylene implants, implanted in patients during femoral-popliteal bypass surgery, noted the patency of prostheses after 3 years in only 58% of patients [6]. As a result, the use of synthetic endoprostheses of the cardiovascular system causes the risk of thrombosis and thromboembolic syndrome and, as a consequence, a decrease in blood flow and death. The highest frequency of such complications and consequences is observed in patients with systemic diseases (diabetes, oncology, etc.), as well as in prosthetics of small-diameter vessels, less than 6 mm.

2. Results and Discussion
In this study, the carbon layer was investigated on vascular polyurethane implants of 10 mm length, 2 mm outer diameter, and 0.1 mm wall thickness. The polyurethane samples were synthesized from a prepolymer based on polypropylene glycol with hydroxyl groups terminated by 2,4-toluene diisocyanate. For synthesis, the prepolymer was mixed with polytetrahydrofuran using an injection molding method to create the desired shape of the prosthesis. After synthesis, the samples were washed in a medical-certified heptane solvent and in deionized water.

The carbon layer on the outer surface of vascular implant was done with using of the plasma-immersion ion implantation (PIII) [7]. The samples were treated with nitrogen ions with an energy of 20 keV from all sides in three steps with a sequential rotation by 120 degrees at each step. For this, the
implants were fixed on the planar high-voltage electrode, where their top side of the implant was only treated at one turn. The processing time at one turn was 800 s, which corresponded to a fluence of \(10^{16}\) ions/cm\(^2\).

However, the ion-plasma system with a planar electrode geometry does not allow treating the inner surface of the implant. A method, where the inner surface of the vessel implant is treated, was developed. A plasma stream is created in a tube between the high-voltage electrode and the grounded wall of the vacuum chamber using a pulsed high voltage. The implant was surrounded with the high voltage electrode where rectangular high voltage pulses of 10 kV with a frequency of 50 Hz and a pulse width of 20 \(\mu\)s were applied (Figure 1). Nitrogen is supplied through the polyurethane tube into the cavity of the implant. The pressure \(P_1\) in the tube and in the implant was 2-3 Torr, which is 4 orders of magnitude higher than the pressure \(P_2\) in the vacuum chamber. This provides a condition for creating a plasma discharge in the implant cavity and its absence in the rest of the vacuum chamber. Thus, only the inner surface of the implant was treated with high-energy ions. The processing time for the inner surface of the implant was 600 s.

The implant becomes dark after the plasma treatment (Figure 2), which is characteristic of the carbonized layer [8, 9, 10]. Fourier infrared spectra of attenuated total reflection (FTIR ATR) spectra were recorded on a Bruker Vertex spectrometer with a spectral microscope to investigate the molecular structure of the surface layer of the implant and the protein attachment. A high sensitivity cadmium mercury telluride (MCT) detector cooled with liquid nitrogen was used. Because the depth of penetration of the infrared beam into the polyurethane is an order of magnitude greater than the thickness of the carbon layer, and the thickness of the protein monolayer by 2 orders of magnitude greater, then the subtraction of the spectra was applied according to the Bouguer-Lambert-Beer law. To analyze the molecular structure of the layer, the spectrum of the original polymer was subtracted. To analyze the protein attachment, the spectrum of the implant without protein was subtracted. The subtracted spectrum was analyzed.

In the subtracted spectrum a new broad band with a centre at 3370 cm\(^{-1}\) of vibrations of hydroxyl and amine groups, a new broad band in the region of 1750-1600 cm\(^{-1}\) of vibrations of double bonds C = O, C = N, and C = C, as well as 1534 cm\(^{-1}\) vibrations of the amine group were observed. Therefore, FTIR ATR spectra showed carbonization, oxidation and amination of the inner surface of the implant as a result of plasma treatment.

Micro-FTIR ATR spectroscopy was used for the adsorption activity of the carbon layer to the tropoelastin protein. The tropoelastin protein was attached to the implant inner and outer surface from a buffer solution. Both treated and untreated implants were used in the experiments. After attachment, the implants were repeatedly washed with a buffer solution to remove all residual not-attached protein, and finally with deionized water to remove salts of the buffer solution. Then the samples were dried in air for 24 hours in air. The micro-FTIR ATR spectra of the implant inner surface were recorded.

According to the results of the experiment, the Amide 1 and Amide 2 lines are observed in the subtracted spectra of the polyurethane implants. The line Amide A was overlapped with own line of the implant and was not taken for the analysis. The spectra showed these lines in treated and untreated polyurethane implants, referring to the vibrations of a group of atoms in the peptide bond of the protein molecule attached to the surface. The intensity of the protein lines in the spectrum of the treated implant is about two times higher than in the spectrum of the untreated implant.

The experiment was repeated with an additional step: washing the polyurethane implants with attached protein in a detergent (sodium dodecyl sulfate) at a temperature of 80 °C for 1 hour. This detergent is used to wash off the covalently unbound protein from the surface [11]. After washing in detergent, the implants were repeatedly washed with deionized water to remove detergent solution. In this case, protein lines were retained in the spectrum of the treated implant, while protein lines were no longer observed in the spectrum of the untreated implant. Thus, the covalent attachment of protein to the plasma-treated inner surface of the implant was confirmed.
Figure 1. Scheme of plasma treatment for inner surface of the implant.

Figure 2. Darkening of the implant after the plasma treatment.

The experimental data of micro-FTIR ATR spectroscopy of the inner surface of shunts made of polyurethane treated with high energy ions are consistent with the results of our previous work on the processing of flat polymer samples [12]. The reason for the covalent attachment of a protein to a carbonized surface is explained by a presence of an unpaired electron stabilized in a $\pi$-electron cloud of carbon clusters [13], the presence of which was confirmed by Raman spectroscopy and electron paramagnetic resonance (EPR) spectroscopy. The presence of unpaired electrons in the carbon layer of the polyurethane implant is observed in EPR spectra. Figure 3 shows the EPR spectrum of a polyurethane implant treated with high-energy ions inside and outside according to the methods described above. These spectra were recorded on an ADANI SpinScan spectrometer at room temperature. For measurements, the polyurethane implant was placed in a quartz capillary, which was located in the sample holder in the cavity between the spectrometer magnets. The spectra were recorded at a magnetic field center position of 333.835 mT, a magnetic field variation range of 10 mT, a modulation frequency of 1 GHz with a modulation amplitude of 300 uT, an electromagnetic field frequency of 9.36 GHz, 1000 scan points, and a signal accumulation time of 240 seconds. The EPR signal showed that in the treated polyurethane implant there are unpaired electrons that have spins...
oriented along the lines of the spectrometer magnetic field. The presence of such unpaired electrons in the polymer is explained by the presence of free radicals on carbon atoms stabilized at the edges of aromatic clusters such as graphite or graphene-like structures [14, 15]. The concentration of free radicals is proportional to the intensity of the spectrum. It can be seen from the spectra that the signal intensity for the treated implant is higher than for the untreated one and goes beyond the noise level. We have to point out, that the EPR spectra were recorded after a year storage of the treated implant under laboratory conditions. Therefore, the free radicals on the carbon clusters are sufficiently stabilised.

Figure 3. Electron paramagnetic resonance spectra of the untreated and treated polyurethane implants. The film was processed and stored for 1 year under laboratory conditions before recording the spectra.

To assess the thrombus formation reaction, the polyurethane implants of blood vessels with the carbon layer and attached protein, as well as untreated polyurethane implants for a control, were implanted into the carotid artery of rabbits (Soviet Chinchilla kind). This experiment has been approved by the Perm Medical State University Local Ethics Committee and conducted in accordance with ICH Harmonized Tripartite Guideline for Good Clinical Practice (ICH GCP). The plasma treated polymeric vascular implants were coated with a layer of tropoelastin protein by immersion in a neutral pH buffer solution. The protein concentration in the solution was 100 μg/ml. A total of 2 plasma treated and 2 untreated implants were used for 4 rabbits. Anticoagulant therapy was performed only at the time of surgery. After the operation, blood anticoagulants were not used. The period of exposure of the implants in the blood stream was a week. After that, the animals were euthanized, the implants with the fragments of the surrounding tissues was excised and fixed in formalin. Then the tissue sample was cut into two parts across the vessel, standard preparation of histological sections was performed, and stained with hematoxylin and eosin. To assess the response, sections were made at both ends of the anastomosis and in the middle of the removed vessel. To obtain a complete picture along the entire vascular bed, a study of blood flow cavities was performed using a microfocus fluoroscopy system with a computed tomography Nikon Metrology XT H 225. Data from both methods are shown in Figures 4 and 5. The mean values of the cross-sectional area of blood flow in vascular grafts were calculated.
Figure 4. The results of the histological study of recanalization in the plasma and protein treated and untreated polyurethane implants.

Figure 5. The results of the tomography study of recanalization in the plasma and protein treated and untreated polyurethane implants.

According to the results of histological and tomographic analysis, a formed thrombus is observed in all vascular implants along the entire length. However, the restoration of blood flow, the so-called recanalization, proceeds faster in the implant with a carbon layer and attached protein. The difference in absolute values of the cross-sectional area of blood flow is due to the fact that the sections were made from separate parts of the vessel, and tomography allows you to estimate the average values of the cross-sectional area of blood flow along the entire length of the prosthesis. Statistical analysis cannot be performed due to the limited number of animals, but the significant difference in the cross-sectional area of the blood flow channels inside the treated and untreated vascular implants shows a positive effect of the plasma treatment.

3. Conclusion
The new method of the plasma treatment for an inner surface of the polyurethane implants makes it possible to form a carbon layer capable of covalently binding protein molecules. Small diameter polyurethane implants with a carbon layer and attached protein show improved antithrombogenicity compared to the untreated implants. The results showed that the small diameter vascular implants
applied without anticoagulants and thrombosis can be developed. Further investigations and developments are required.

Acknowledgments
V.S. Chudinov thanks Prof. M. Bilek, Prof. D. McKenzie and Prof. A. Weiss for their permission to work in their laboratories and for provided tropoelastin protein. Further investigations and developments are required.

References
[1] Bussooa A, Neale S and Mercer J 2018 Future of Smart Cardiovascular Implants Sensors 18 2008 Online: http://dx.doi.org/10.3390/s18072008
[2] Mathers CD and Loncar D 2006 Projections of Global Mortality and Burden of Disease from 2002 to 2030 PLoS Med 3 e442 Online: https://doi.org/10.1371/journal.pmed.0030442
[3] GBD 2016 Causes of Death Collaborators 2017 Global, regional, and national age-sex specific mortality for 264 causes of death, 1980–2016: a systematic analysis for the Global Burden of Disease Study 2016 The Lancer 390 1151–210
[4] Ward WK 2008 A review of the foreign-body response to subcutaneously-implanted devices: the role of macrophages and cytokines in biofouling and fibrosis J. Diabetes Sci. Technol. 2 768–77
[5] Hu WJ, Eaton JW, Ugarova TP and Tang L 2001 Molecular basis of biomaterial-mediated foreign body reactions Blood 98 1231–38
[6] Haimov H, Giron F and Jacobson JH 1979 The Expanded Polytetrafluoroethylene Graft. Three Years' Experience with 362 Grafs Arch. Surg. 114 673–9
[7] Anders A 2000 Handbook of Plasma Immersion Ion Implantation and Deposition (NY: Wiley)
[8] Dong H and Bell T 1999 State of the art overview: ion beam surface modification of polymers toward improving tribological properties Surface and Coatings Technology 111 29–40
[9] Odzhaev VB, Kozlov IP, Popok VN and Sviridov DB 1998 Ion Implantation of Polymers (Minsk: Belorussian State University)
[10] Fink D 2004 Fundamentals of Ion-Irradiated Polymers (Berlin: Springer)
[11] Kiae D, Hoffman A S and Horbett TA 1992 Tight binding of albumin to glow discharge treated polymers J Biomater Sci Polym Ed 4 35–44
[12] Chudinov V, Kondyurina I, Terpugov V and Kondyurin A 2019 Weakened foreign body response to medical polyurethane treated by plasma immersion ion implantation Nucl. Instrum. Methods Phys. Res. B 440 163–74
[13] Kondyurin A and Maiz M 2007 Surface modification of ePTFE and implants using the same US patent 2007/005007 A1
[14] Emanuel NM and Buchachenko AL 1988 Chemical Physics of Molecular Decomposition and Ageing of Polymers (Moscoc: Nauka)
[15] Gambino RJ and Thompson JA 1980 Spin resonance spectroscopy of amorphous carbon films Solid State Commun. 34 15–18