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Cell growing on ion implanted polytetrafluorethylene

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ABSTRACT

Polytetrafluorethylene (PTFE and ePTFE) substrates were treated by ion implantation with nitrogen ions of 20 keV energies and 10^{13}–10^{16} ions/cm^2 ion fluences. The modification of the polymer surface was analyzed by FTIR and XPS spectroscopy, water wetting angle measurements and AFM images. The surface morphology, wettability and chemical activity were changed due to surface modification. The growing of endothelial cells of modified surfaces was improved in comparison with untreated PTFE and ePTFE substrates. The improved cell adherence on the modified surface is based on the improved adhesion of cell proteins.

1. Introduction

Polytetrafluorethylene (PTFE) is known for its excellent chemical and bio stability and its bio inertness. The mechanically stretched, expanded form (ePTFE) is microscopically porous, possessing otherwise the properties of standard PTFE [1]. ePTFE found a large number of applications in surgery as flaps in herniorrhaphy, craniosurgery, etc., mainly because of its bioinertness, biostability, both flexibility and mechanical stability. However, a main domain is in vascular surgery for as prostheses for smaller, i.e. infrainguinal blood vessels, artificial AV shunts, as coating of vascular stents or heart valve rings.

Despite of wide applications, there are still two main problems with ePTFE implant: thrombosis and anastomosis stenosis by intima hyperplasia. The hemodynamic and a mismatch of the elastic properties of the graft and native vessel were considered as reasons for graft failure [2]. These problems could not be solved by improved surgical techniques but are considered as materials related. Especially thin vessel grafts are susceptible for these problems.

The extreme hydrophobicity of ePTFE and the low toxicity is regarded as the reason for the good performance in bigger vessels, but a superhydrophobic modification of ePTFE failed for small diameter grafts and in bigger vessels it showed a higher platelet adsorption than standard ePTFE grafts [3]. Additional surface coating with biologically designed surfaces such as heparin or hirudin can reduce platelet adherence and the intima proliferation but has the problem of a physiological decay in biological activity and in some cases iatrogenic inactivation by protaminsulfate [4–6].

Endothelial cells as the inner lining of blood vessels are known to provide the best hemocompatible surface. All PTFE-like materials do not support endothelialisation in vivo [7]. There is a concept is in vitro tissue engineering, seeding of autologous endothelial cells on the implant surface prior to implantation [8]. Various attempts have been made to achieve an adherent layer of endothelial cells on the surface. Seeding the cells in a system with dynamic pressure and flow [9,10] has been described as the easiest way to obtain an endothelial lining, which resists the shear stress of physiological blood flow, an effect which also works in other systems [11].

Some ways of surface modification of ePTFE have been successfully attempted based on plasma method of modification [12]. An improved cell adherence and proliferation over untreated ePTFE was found as graft for intracranial arteries. In the latter case an increased adhesion of fibrin-glue was found, which allows better anchorage of the flap. The trend is to surface modification, which provides specific cell adherence. These cells adhere via protein receptors at the extracellular matrix (ECM) and their basement membrane. The native extracellular matrix

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[13] or selected ECM proteins, collagen [14], fibronectin [15], and laminin [16] should be adsorbed on the ePTFE surface. The interaction of these proteins with the integrin is provided by a special, highly conservative functional group of the ligand, the RGDS sequence as well as a peptide of this individual sequence should be immobilized [15]. In all cases endothelial cells were seeded in vitro and an improved growth of the cells have been demonstrated.

The preparation of PTFE surface for the increased growth of endothelial cells can be achieved by ion beam implantation [17,18]. The method is based on structural modification of a thin surface layer of a polymer under the bombardment by high energy ions [19–21]. The effect of the modification is caused by the penetration of the high energy ion into the polymer, cascades of collisions with atoms of macromolecules and transfer of the kinetic energy of penetrating ion to atoms and electrons of polymer macromolecules. The transferred energy is high enough that atoms and electrons leave macromolecules and fly away with high kinetic energy causing new collisions with nearest macromolecules. As result, breaking of chemical bonds, ionization, formation of free radicals, electron and phonon excitation of macromolecules are observed [22]. The field of such strong structure changes of polymer is named an ion track and the size of ion track depends on ion energy, kind of ion and polymer. This is a first stage of the ion beam implantation of polymers.

After ion penetration, the track field of polymer has a very high concentration of free radicals, ionized and highly excited parts of macromolecules. These active particles cause a number of chemical reactions. As result, amorphous carbon, aromatic condensed structures, stable and semi-stable free radical structures appear. The properties of the polymer surface after ion beam implantation are mostly connected with this second stage. The morphology of the surface changes dramatically with formation of rough structures [23]. The water contact angle of PTFE and ePTFE surface after modification is observed higher [24] and lower [25], than the initial. The vibrational spectra show a number of new chemical groups [26].

In general, the kinetics of free radicals takes sufficiently long time (from minutes to days) then the first stage (part of second) due to long kinetics of free radical reactions in PTFE [27,28]. The presence of free radicals in PTFE causes chain reactions of fluorine cleaving from initial macromolecules, chain breaking and crosslinking [29–31]. Due to migration of free radicals, the modified area can be shifted to significantly deeper layers then the track of ion [32]. In air the free radical reactions of modified polymer layer are carried out with participation of oxygen when the stable oxygen-containing groups appear in polymer [28]. Such modified polymer surface is used for different applications [33] including medical devices [21,34–36]. However, the structure transformation of ion implanted PTFE and ePTFE and a biological response on the modified PTFE have not been fully investigated as required for medical applications.

In present investigation the ion implantation was applied to PTFE and ePTFE surfaces. The structure changes in PTFE and ePTFE after ion implantation were investigated and linked to the improved cell adherence.

2. Experiment

ePTFE sheets were provided by Boston Scientific SCIMED, USA. PTFE films of 20 μm were provided by Halogen, Perm, Russia. The PTFE films were cleaned by ethanol and dried before using. The ePTFE samples were used as provided and handled between protective coatings to prevent contamination of the surface. Acrylamide with 0.1% of Tetramethylethylenediamine inhibitor of polymerization (Aldrich) was dissolved in deionized water with a concentration of 100 g/L.

Ion implantation of PTFE was done on Pulsar implanter described in [37]. The beam of nitrogen ions with 20 keV energy, current density of 5 mA/cm^2 in a pulse of 300 ns duration with pulse-repetition frequency of 1 Hz was applied. The fluence of ions was measured with Faraday-cup.

For acrylamide attachment the modified samples were soaked in 10% acrylamide water solution during 2 h at room temperature. After soaking the samples were washed by deionized water 3 times and dried on air overnight.

Plasma immersion ion implantation was used for modification of the PTFE and ePTFE substrates. The samples were placed on the high voltage electrode and covered by stainless steel mesh. The distance between mesh and sample surface was 30 mm. The mesh had the same electrical potential as electrode. The plasma chamber was vacuumed up to 10^{-3} Pa and filled by nitrogen up to 5×10^{-1} Pa. Plasma discharge was generated by RF-frequency generator of 200 W power at 13.75 MHz frequency. The high voltage pulsed biases of 20 kV was used. The pulse of 5 μs duration with 50 Hz frequency of repetition was applied to high voltage electrode. Ion fluence estimates for the plasma immersion ion implantation were obtained in an experiment on polyethylene using a procedure described in [21]. The fluence of one high voltage pulse was determined by comparing UV transmission spectra from polyethylene films after the plasma immersion ion implantation to samples implanted with known ion fluences in Pulsar ion implanter experiments where the fluence was measured with a Faraday cup. The pulse duration, pulse repetition frequency, average current, kind and energy of ions were adjusted to match the both experiments. One high voltage pulse of the plasma immersion ion implantation under the present conditions was found to correlate with a fluence of 2.8×10^{11} ions/cm^2. The PTFE and ePTFE samples were treated with 35–35,000 pulses, corresponding to implantation ion fluences of 10^{13}–10^{16} ions/cm^2. The plasma immersion ion implantation of PTFE has shown similar results as for ion implantation of PTFE with Pulsar implanter.

Fourier transform infrared attenuated total reflection (FTIR ATR) spectra were recorded on Nicolet Magna spectrometer with Ge ATR crystal with 45° of incident angle. Number of scans was 100, spectral resolution was 2 cm^{-1}.

The contact angles between a PTFE and ePTFE sample surface and de-ionized water were measured using a Kruss contact angle analyzer DS10 employing the sessile drop method.

X-ray photoelectron spectra (XPS) were recorded on Scanning Auger electron spectrometer Microlab 310F (Fisons) with accessory XPS-unit (Al/Mg – X-ray tube). Scanning step was 0.2 eV.

An atomic force microscope DI-3100 (Digital instruments Ins., Santa Barbara) was used for surface morphology analysis of the PTFE samples before and after ion implantation. The measurements were carried out in a tapping mode. Silicon probes with 75 kHz resonant frequency, 1.5–3.7 N m^{-1} spring constant and 10 nm curvature radius were used. The initial PTFE film was too rough for AFM measurement. To get smooth PTFE surface, the initial PTFE film was pressed between Silicon wafers before the ion implantation. A surface topology of the PTFE film after pressing was similar to the silicon wafer surface topology.

In cells experiment, ePTFE discs with the surface area of 0.58 cm^2 were mounted in Minusheets (Minucells and Minutissue, Bad Abbach) and steam sterilized at 120 °C. The bovine aortic endothelial cell line GM7373 (Coriell) was used for the experiments [38]. 4×10^4 cells in 200 μL medium (MEM-Earle supplemented with 10% fetal bovine serum (FBS), 1 × MEM vitamins, 2 mM N-acetyl L-alanyl L-glutamine, 1 × amino acids) were seeded directly on the samples. They were allowed to adhere for 2 h at standard cell culture conditions, then medium was filled up to 1 mL per sample. 3