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Athrombogenic hydrogel coatings for medical devices – Examination of biological properties

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ABSTRACT

In the article the authors present hydrogel coatings prepared from polyvinylpyrrolidone (PVP) macromolecules, which are chemically bonded to polyurethane (PU) substrate. The coating is designed to improve the surface hemocompatibility of blood-contacting medical devices.

The coating was characterized in terms of physical properties (swelling ratio, hydrogel density, surface morphology, coating thickness, coating durability). In order to examine surface hemocompatibility, the materials were contacted with whole human blood under arterial flow simulated conditions followed by calculation of platelet consumption and the number of platelet aggregates. Samples were also contacted with platelet-poor plasma; the number of surface-adsorbed fibrinogen molecules was measured using ELISA assay. Finally, the inflammatory reaction after implantation was assessed, using New Zealand rabbits.

The designed coating is characterized by high water content and excellent durability in aqueous environment – over a 35-day period, no significant changes in coating thickness were observed. Experiments with blood proved twice the reduction in adsorption of serum-derived fibrinogen together with a moderate reduction in the number of platelet aggregates formed during the contact of the material with blood. The analysis of an inflammatory reaction after the implantation confirmed high biocompatibility of the fabricated materials – studies have shown no toxic effects of the implanted material on the surrounding animal tissues.

1. Introduction

The formation of thrombus on biomaterial surface still remains a serious problem during the applications of implants controlling the blood flow, i.e. in mechanical heart assist devices. The highly adverse and dangerous effects include clot formation inside the prosthesis cavity, followed by the blood clots release to the patient's arteries, which entails the risk of serious complications for the patient's life and health. Another undesirable effect is platelets contact activation, resulting in platelets micro-particles distribution to the patient's circulation, playing significant role in late blood coagulation activation in the patient's peripheral vessels. To prevent blood-clotting process and improve the prostheses hemocompatibility, various methods of surface modifications have been proposed.

It is worth noticing, that the layer of proteins adsorbed on the surface of the material determines all interactions between the biomaterial and various blood components. The number, type and spatial conformation of the adsorbed proteins is, in turn, determined by the physicochemical properties of the surface material, in particular its wettability, morphology and chemical composition. There are many reports on the influence of the surface wettability on the process of protein adsorption and cell adhesion [1–4]. Generally, it is believed that surfaces with low wettability (hydrophobic) promote the adhesion and conformational changes of proteins, whereas the surfaces with high wettability (hydrophilic) reduce protein adsorption and minimize the possibility of denaturation. Therefore, the majority of modification methods are based on increasing the hydrophilicity of the material. One of the techniques for biocompatibility improvement is based on biopassive surfaces

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formation. Biopassive surfaces are characterized by low interfacial energy, which results in a decreased number of surface-adsorbed proteins and surface-adhered cells. In addition, the strength of interaction between the protein and the surface is small, which limits the conformational changes occurring in the protein molecules and enables maintenance of their biological activity [5,6].

As a result of our study, we have developed the process of fabrication hydrogel coatings on the surface of polyurethanes (PU). The coating aims to increase the hydrophilicity of the basis polymer and create a biopassive surface. As the coating materials we used polyvinylpyrrolidone (PVP). PVP displays a high biocompatibility [7–9], antibacterial activity [10,11] and high hemocompatibility – the polymer was used as a human plasma substitute during World War II [12]. Further studies have shown that supplementation with PVP biomaterials can lead to a reduction in hemolysis of erythrocytes [13] as well as a reduction in adsorption of albumin [14], fibronectin [15] and fibrinogen [16]. It has been proven that the PVP coating significantly improves the hemocompatibility of the intravascular catheters [15] and membranes for plasma separation [17]. The great advantage of PVP is its resistance to hydrolysis, which allows to apply it in contact with highly hydrolytic environment such as blood.

In our previous papers, we described the method of coating fabrication and explained in detail the mechanism of hydrogel formation [18,19]. The coating is formed due to free radical macromolecular grafting – crosslinking. Polymer surface was first immersed in an organic solution containing radical source: cumene hydroperoxide (CHP) with addition of a branching and anchoring agent: ethylene glycol dimethylacrylate (EGDMA). In the second step, the substrate was immersed in a water solution containing given concentration of PVP and Fe^{2+}. The novelty of the process consists in the fact that free radicals are formed mostly at the polymer/solution interphase, what assures high grafting efficiency together with formation of covalent bonds between polymer substrate and modifying layer.

In this paper, we present the results of testing the chosen physicochemical and biological properties of the material.

2. Materials and methods

2.1. Materials

Polyurethane (ChronoFlex C, 75D, AdvanSource Biomaterial, Wilmington, U.S.) was purchased in the form of pellets. Reagents, namely polyvinylpyrrolidone (PVP) powder with average molecular weight of 360 kDa, iron (II) chloride ($FeCl_2$), ascorbic acid (AA), cumene hydroperoxide (CHP), ethylene glycol dimethacrylate (EGDMA) and sodium dodecyl sulfate (SDS) were obtained from Sigma–Aldrich (Poznań, Poland). Hexane was purchased from Chempur, Poland.

2.2. PU film preparation

PU disc were injection-moulded from PU granulates to a form of discs with 40-mm diameter. The discs were cut out with a metal stamp and subsequently modified as follows. Before modification PU discs were washed with 5% alcohol solution and dried.

2.3. PU modification with hydrogel coating

PU samples were modified according to the previously presented free-radical-based method [18]. Briefly, the PU discs were immersed in a hexane solution containing EGDMA (5%, v/v) and CHP (3%, v/v) for 5 min at RT. The samples were then placed in a water solution containing given amounts of PVP (5%, w/v, for PVP_5 or 10%, w/v, for PVP_10), $FeCl_2$ (0.1%, w/v) and 0.1% (w/v) AA for 15 min at RT. After the coating procedure, the polymer discs were washed with 0.1% (w/v) SDS water solution for 5 min and subsequently in water (overnight).

In order to compare coatings fabricated with the proposed method, the control samples (PVP_S) were also prepared. These samples were fabricated using standard dip-coating technique, without the presence of free radicals or cross-linking agent in the coating solution. PU discs were immersed in 10% solution of PVP for 15 min and subsequently washed with 0.1% (w/v) SDS water solution for 5 min.

2.4. Coating characterization

Physical properties of hydrogel coatings were evaluated by measuring the equilibrium swelling ratio (EWC, %) and hydrogel density (HG, g/m^2) as described previously [18].

The coating thickness and adhesion to the substrate were analyzed microscopically: materials were incubated in PBS at 37 °C to obtain the equilibrium hydration followed by microscopic analysis of cross-sections. To ensure a better visualization of the coating, the samples were stained with rhodamine (0.002%, w/v). Coating thickness measurements were carried out for six replicates of the material, the thickness of each coating was measured at four randomly selected locations. The morphology of the freeze-dried hydrogel coatings was analyzed using a scanning electron microscope (Phenom, Phenom World).

2.5. Coating durability

The stability of hydrogel coating in an aqueous medium was determined by the analysis of changes in coating thickness. The samples were incubated in PBS with 0.5% (w/v) sodium azide at 37 °C. After a certain time of degradation (7, 14, 21 and 28 days), the samples were removed from the solution, rinsed with PBS and the cross-sections were analyzed using optical microscopy. Coating thickness measurements were carried out for six replicates of the material, the thickness of each coating was measured at four randomly selected locations.

2.6. Fibrinogen adsorption

The plasma-derived fibrinogen adsorption to the test materials was analyzed using platelet poor plasma (PPP). In order to prepare PPP, 50 ml of blood was drawn from a healthy, aspirin-free donor (female, 28 years) in the K2EDTA tubes using the BD Vacutainer vacuum system. Blood was centrifuged at 300 × *g* for 30 min. The obtained supernatant was transferred to clean tubes and centrifuged at 2000 for 20 min. After the centrifugation, the supernatant (PPP) was transferred to a new sterile tube.

The samples (disks with a diameter of 18 mm) were placed in 24-well polystyrene plates and equilibrated with PBS at 37 °C overnight. Next, the investigated materials were contacted with 100% PPP for 1 h at 37 °C. Materials with surface-adsorbed fibrinogen were analyzed with ELISA assay. The following procedure was applied: after incubation with PPP, the samples were rinsed (3× 5 min) with washing buffer (PBS supplemented with 0.05% Tween 20, Sigma–Aldrich, Poland), blocked with non-fat dry milk (5% solution in PBS, 1 h, RT), rinsed with washing buffer (3× 5 min), incubated with primary antibodies (1 h, RT), rinsed with washing buffer (3× 5 min), incubated with secondary antibodies (1 h, RT), rinsed with washing buffer (6× 5 min) and transferred to fresh plates to eliminate the influence of the protein adsorbed to the well walls. Materials were then incubated with peroxidase substrate solution – SigmaFast OPD (*o*-phenylenediamine dihydrochloride, Sigma–Aldrich, Poland) in the dark at RT for 30 min. After the reaction, a part of the solution (200 μl) from each well was transferred