Colloids and Surfaces B: Biointerfaces 144 (2016) 335–343

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

Endothelialization of polyurethanes: Surface silanization and immobilization of REDV peptide

Beata A. Butruk-Raszeja[a,*], Magdalena S. Dresler[a], Aleksandra Kuźmińska[a], Tomasz Ciach[a,b]

[a] Laboratory of Biomedical Engineering, Faculty of Chemical and Process Engineering, Warsaw University of Technology, Warynskiego 1, 00-645 Warsaw, Poland
[b] CEZAMAT PW, Polna 50, 00-644 Warsaw, Poland

ARTICLE INFO

Article history:
Received 8 October 2015
Received in revised form 5 April 2016
Accepted 7 April 2016
Available online 8 April 2016

Keywords:
REDV
Endothelial cells
Polyurethane
Blood compatibility
Platelet adhesion
Surface endothelialization

ABSTRACT

The paper presents method for chemical immobilization of arginine-glutamic acid-aspartic acid-valine (REDV) peptide on polyurethane surface. The peptide has been covalently bonded using silanes as a spacer molecules. The aim of this work was to investigate the proposed modification process and assess its biological effectiveness, especially in contact with blood and endothelial cells. Physicochemical properties were examined in terms of wettability, atomic composition and density of introduced functional groups and peptide molecules.

Experiments with blood showed that material coating reduced number of surface-adhered platelets and fibrinogen molecules. In contrast to polyurethane (PU), there were no blood components deposited on REDV-modified materials (PU-REDV); fibrinogen adsorption on PU-REDV surface has been strongly reduced compared to PU.

Analysis of cell adhesion after 1, 2, 3, 4, and 5 days of culture showed a significant increase of the cell-coated area on PU-REDV compared to PU. However, an intense cell growth appeared also on the control surface modified without the addition of REDV. Thus, the positive effect of REDV peptide on the adhesion of HUVEC could not be unequivocally confirmed.

1. Introduction

Despite the huge advances in the development of biomaterials, resulting in a significant improvement in biocompatibility of synthetic materials, nature remains irreplaceable. Still, endothelium remains the only athrombogenic and self-healing surface. Therefore, the latest trend in the area of vascular biomaterials has focused on restoring the endothelial monolayer by designing the cell-selective surfaces with specific biological properties. This solution is close to ideal and leads to the creation of hybrid implants – synthetic structures covered with patient cells that actively prevent blood clotting processes and provide conditions similar to those inside blood vessels.

Surface endothelialization of vascular prosthesis may be performed in two ways: *in vitro* and *in situ*. *In vitro* strategy is based on the classic tissue engineering scheme comprising cell sampling followed by *in vitro* proliferation and culturing on the surface of the prosthesis. The cell-scaffold construct is then implanted into the patient body. *In vitro* endothelialization process is burdened with the risk of infection and the need for providing cell culture, which significantly increases time and costs of the process. The situation has improved with the discovery of a new population of endothelial progenitor cells (EPCs). These cells are located mainly in the bone marrow, but also circulate in small concentrations in peripheral blood, where they are involved in the regeneration of damaged blood vessels [1–3]. This discovery was used to develop a strategy for *in situ* endothelialization. The idea of this process is to place the prosthesis with a suitably modified surface inside the patient's body. Such prostheses capture endothelial progenitor cells from the blood and promote the process of adhesion, proliferation and differentiation of EPCs into mature endothelial cells. The process of *in situ*

* Corresponding author.
E-mail address: b.butruk@ichip.pw.edu.pl (B.A. Butruk-Raszeja).

http://dx.doi.org/10.1016/j.colsurfb.2016.04.017
0927-7765/

endothelialization can restore the endothelium structure without introducing cells into a patient, minimize the risk of infection and immune response and eliminates the need for immunosuppressive drugs application.

The process of surface endothelialization relies on the interaction between membrane receptors on the cell surface and their specific ligands present on the surface of the biomaterial. Cell receptors recognize peptide sequences, therefore, it is necessary to modify the surface of the polymer with biomolecules such as proteins or peptides. Immobilization of biomolecules must be stable and, at the same time, the process cannot change their natural conformation, as this would lead to a loss of biological activity of the introduced biomolecules. Submitted biomolecules must also be selective for endothelial cells and promote their adhesion, but at the same time they must not cause the adhesion of other cells present in the blood. Selectivity of the introduced biomolecules is particularly important in the process of *in situ* endothelialization, in which EPCs bind directly from the bloodstream. As already mentioned, the concentration of EPCs in peripheral blood is extremely low. Therefore, any competition from other blood cells in the process of adhesion would significantly reduce the effectiveness of surface endothelialization. In addition, selectivity of the surface biomolecules is crucial in terms of biomaterial hemocompatibility. In the case of prosthesis' endothelial monolayer damaged, the surface should not promote adhesion of other cells, particularly platelets, as it could lead to activation of blood clotting process.

The development of bioactive surface enabling endothelialization process would be a tremendous step forward in cardiovascular engineering. However, the main problem that remains unsolved is finding the appropriate surfaces that are able to selectively "capture" endothelial cells from the bloodstream and to support the processes of adhesion and proliferation. The first attempts to culture endothelial cells on polymeric substrates were based on the use of substrates with appropriate wettability [4]. Next, various methods of covalent immobilization of extracellular proteins, mainly fibronectin [5], fibrinogen [6], and collagen [7] have been proposed. At this point it is worth noting that immobilization of proteins on the prosthesis' surface is associated with many difficulties. First, there is a risk of an immune response from the patient. Second, the immobilized proteins exhibit high instability in contact with the culture medium and can quickly degrade in the aqueous environment [8]. It was also shown that the covalent bonding of proteins may lead to a reduction in their biological activity [9]. It is absolutely vital to mention the risks associated with blood clotting – matrix proteins have a high thrombogenic potential and can activate coagulation processes in contact with the patient's blood.

The best solution seems to be the application of short peptide sequences recognized by cell receptors. Peptides are more stable compared to the proteins, both in chemical modification processes as well as during contact with patient's body fluids [10]. So far, the most commonly used peptide was the tripeptide arginine-glycine-aspartic acid (RGD). This peptide has been used successfully for surface endothelialization [11–13]. However, it should be noted that RGD is also recognized by platelet integrin receptors which can promote their adhesion [14].

In summary, many surfaces promoting adhesion of endothelial cells have been developed. However, most of them not only promote the adhesion of endothelial cells, but also interact with other types of cells and platelets. Therefore, current research on endothelialization is centered on finding the suitable capture molecule. Recent reports suggest a high selectivity of a tetrapeptide arginine-glutamic acid-aspartic acid-valine (REDV) toward human endothelial cells [15–19]. The aim of presented work was to develop technology for introduction REDV peptides on polyurethane surfaces and analyze biological properties of the modified materials. The results of material hemocompatibility studies as well as endothelial cell adhesion analysis are presented herein.

2. Materials and methods

2.1. Material preparation

2.1.1. Polyurethane film preparation

PU films were prepared by solution casting and solvent evaporation technique. Briefly, polyurethane pellets (ChronoFlex, AdvanSource Biomaterials, USA) were washed with alcohol/water solution, dried and dissolved in DMAC (Sigma-Aldrich, Poland) at a concentration of 20% w/v. The solution was poured onto a flat clean glass and dried at 37 °C until total solvent evaporation. The obtained film was peeled-away, cut and used for further modification. In order to chemically immobilize the peptide on the polyurethane surface, multistep modification was conducted. The steps were as follows: surface activation, introduction of spacer molecules and peptide coupling.

2.1.2. Surface activation

Polyurethane surface were activated using silicone (IV) chloride (STC, Acros Organics). A solution of 1% SiCl4 in anhydrous toluene was prepared. Anhydrous toluene was obtained from standard solvent by dehydration with molecular sieves (3A, Sigma-Aldrich, Poland) for 24 h. PU samples were immersed in the solution for 5 min, rinsed with anhydrous toluene and left for a few minutes to air-dry. Next, samples were put inside closed containers filled with water for 1 h in 30 °C in order for hydrolysis to occur. In the end, all silanized samples (PU-OH) were rinsed with anhydrous toluene and left for air-dry.

2.1.3. Introduction of spacer

(3-Aminopropyl)triethoxysilane (APTES, Sigma-Aldrich, Poland) was chosen as a spacer molecule. A solution of 96% alcohol was prepared (pH adjusted to 5 with acetic acid) and APTES at a concentration of 2% v/v was added. The solution was left for 5 min for free silanols formation. Silanized samples (PU-OH) were immersed in the solution (separately, in closed containers) and left for 15 min. After that time materials were rinsed with alcohol solution, and then twice with water (2 × 15 min, on stirrer). Finally, aminated samples were dried in 40 °C overnight. To convert terminal amine groups to carboxyl groups, materials were reacted with glutaric anhydride (1% in phosphate buffer pH = 7, Sigma-Aldrich, Poland) for 1 h, followed by rinsing with water.

2.1.4. Peptide coupling

Peptide coupling to carboxylated surfaces was conducted using sulfoNHS/EDC chemistry. First, samples were activated in 0.1 M MES buffer (pH = 6) for 1 h. Next, materials were immersed in a solution of 5 mM sulfo-NHS (Sigma-Aldrich, Poland) and 2 mM EDC (Sigma-Aldrich, Poland) for 15 min at room temperature. Subsequently, peptide coupling was conducted. REDV peptide was dissolved in a phosphate buffer (pH = 8.0) in proper concentration (5 mM); samples (PU-COOH) were immersed in peptide solution and left for 24 h at room temperature. Next, materials were rinsed with washing buffer (PBS-Triton; Sigma-Aldrich) followed by washing with PBS buffer.

2.2. Physicochemical properties

2.2.1. XPS analysis

The changes of chemical composition during surface modification were determined by XPS on a VG Scientific ESCALAB photo-electron spectrometer. Spectra fitting and determination of