Fabrication of novel material with athrombogenic potential: Immobilization of peptides on polyurethane surface

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Presented study was undertaken to fabricate a hemocompatible material that could be a potential candidate for use in fabrication of blood-contacting devices, e.g. circulatory support devices, vessel grafts, heart valves. The final material could be used as a scaffold for endothelial cell growth and initiate in situ endothelialisation. The newest and most promising strategy in material endothelialization involves introduction of short peptide sequences that can selectively address one particular type of cell adhesion receptors and promote cell adhesion. Thus, the aim of work was to elaborate a method of immobilizing peptides specific for integrins onto polymer substrate.

Keywords and phrases: polyurethane, endothelium, peptides immobilization.

Introduction

Among the major interests of modern biomedical engineering is to construct a heart prosthesis that would not require any intervention after implantation. The inner side of such an artificial heart needs to be covered by a material that would mimic a natural bloodcontacting surface. Implantable material should avoid unwanted phenomena such as inflammation and blood coagulation, which happens *in vivo* on unprotected artificial surfaces. Usually, a polymer such as polyurethane or poly(tetrafluoroethylene) undergoes further chemical or physical modification. A number of methods have been applied: hydrophilization [1] or hydrophobization [2] of a polymer surface and immobilization of biomolecules such as phospholipids [3] or heparine [1]. The most advanced method is to cultivate endothelial cells on previously prepared polymer surface.

Endothelial cells exist inside natural vessels and heart and play a major role in interaction between blood and a blood — contacting surface. Among others, those cells provide vital features as haemostasis control, protecting from trombosis and neointimal hyperplasia [4]. A variety of strategies has been used to promote the endothelialization of synthetic surfaces [5–6]. However, polymers generally used in implantation are bioinert, which means that cells cultivation does not occur spontaneously on their surface. Our assumption was to promote cell proliferation by enriching polyurethane surface with short peptides that are specific to cell membrane receptors — integrins. The idea of binding peptides to the polymer carrier through multi-step chemical synthesis has been proposed before [7, 9–10]. In our research we selected methods used in each stage and chose parameters (temperature, time, concentration) that are the most appropriate for used type of polyurethane.

Integrins are cell receptors — proteins that exist in endothelial cell membrane. They are capable of binding specific molecules (ligands) that are found outside a cell. Binding a ligand by an integrin gives a cell critical signals about its surrounding. Depending on what kind of ligand has been bound, integrins trigger biological actions, such as attachment, movement, death, or differentiation. It has been reported that peptides with RGD sequence promote cell adhesion rather than other actions (Fig. 1). To enhance endothelial cell attachment on the polyurethane surface, we have to immobilize short peptides including RGD sequence (arginine--glycine-aspartic acid, figure 2) [7–8]. Recently there is a great interest in arginine-glycine-aspartic acid tripeptide (RGD), which is a minimal binding domain of

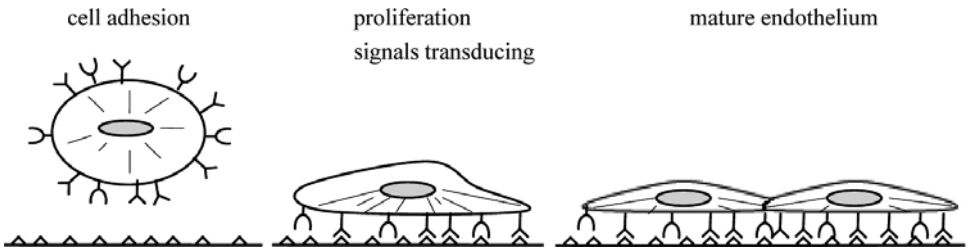

Fig. 1. Integrins — mediated endothelial cells adhesion on the surface covered with peptides — ligands for integrins.

fibronectin and has been studied for its ability to enhance endothelial cell adhesion by receptor-ligand interactions.

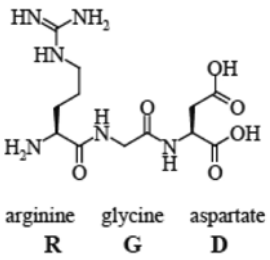

Fig. 2. Structure of RGD — the smallest peptide recognized by integrin.

Materials and methods

As a backbone for further modification polyurethane (PU) was used — namely: Walopur 2102 A in form of foil (0,7 mm thick) supplied by Epurex Films. Following reagents were used: hydrogen peroxide solution (H_2O_2, 30%, provided by Carlo Erba), ammonium cerium (IV) sulphate dihydrate ($Ce(NH_4)_2(SO_4)_2 \cdot 2H_2O$, Riedel — de Haën), nitric acid (HNO_3, 65%, Lach-Ner s.r.o.), acrylic acid ($CH_2CHCOOH$, Fluka), Toludine Blue O ($C_{15}H_{16}ClN_3S$, Sigma Aldrich), sodium hydroxide (anhydrous pellets, Carlo Erba).

The aim is to immobilize tripeptide RGD on polyurethane surface by creating covalent bonds between the two components. To achieve this goal, we proposed grafting a carboxyl containing compound onto polymer surface. Obtained carboxyl groups can easily react with amino acids. Figure 3 illustrates a procedure of acrylic acid grafting. Polyurethane is chemically inert, therefore in the first step activation of its surface is needed. UV light was used to enrich polymer surface with hydroxyl and hydroperoxide groups [9–10]. Fragments of PU foil were immersed in 30% H_2O_2 solution and kept under UVC radiation, then washed with destilled water. Obtained hydroxyl groups are able to react with acrylic acid through radical reaction catalyzed with the oxidation

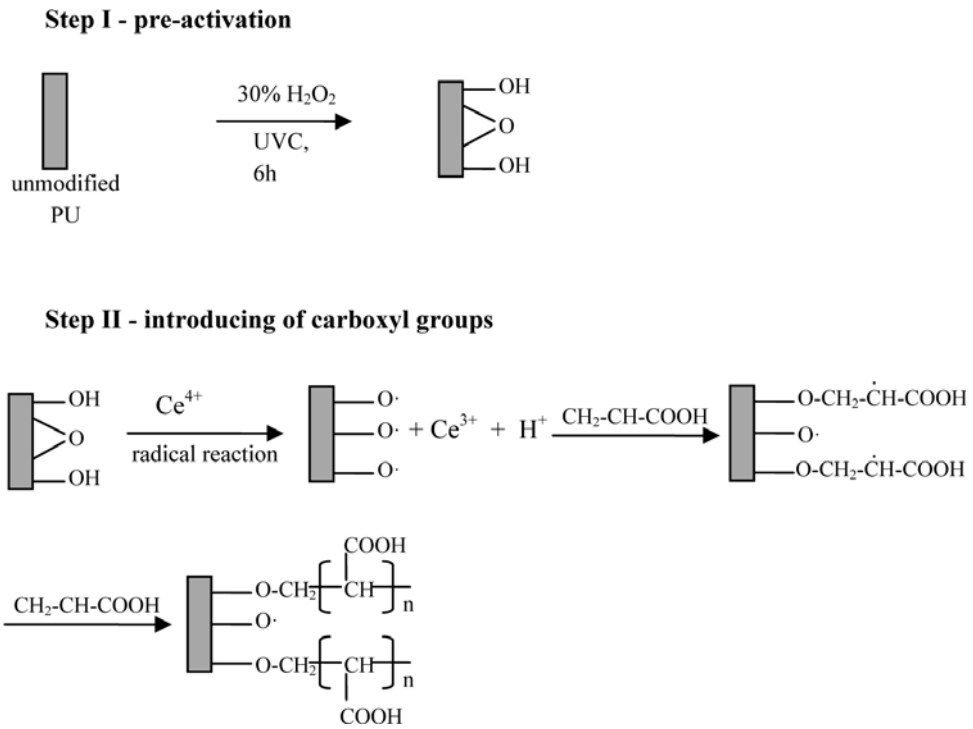

Fig. 3. Acrylic acid grafting procedure.

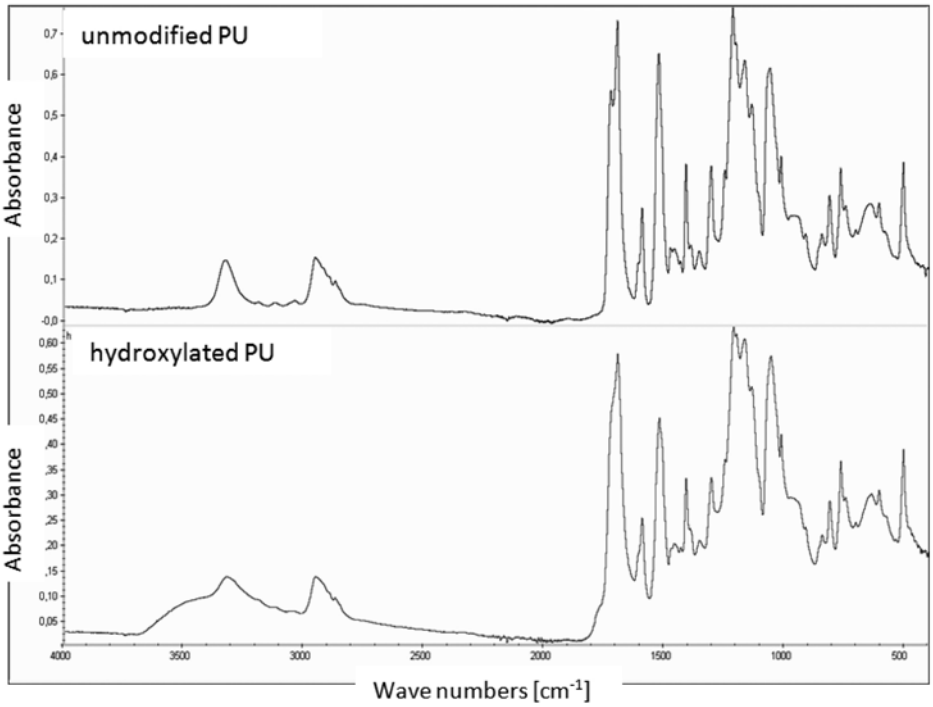

Fig. 4. FTIR-ATR spectra of unmodified PU and PU after 6h of hydroxylation.

of ceric ions [9]. Grafting of acrylic acid was accomplished by immersing UV-treated PU foil in an aqueous solution containing given amount of acrylic acid, 1,50% (v/v) nitric acid and 0.1% (w/v) ceric ammonium sulphate. A set of experiments was carried out to investigate the process of grafting. Tested parameters were: temperature — 25, 35, 40 and 45°C, reaction time — 0,5, 1,5 and 2,5h and concentration of acrylic acid — 5, 10, 15 and 20% (v/v).

Next step in not yet accomplished due to relatively short time of research and will be proceeded soon. Here, a peptide bond would be formed between carboxyl groups present on polymer surface and the first amino acid — arginine. We suggest the use of carbodiimide (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide), EDC). EDC coupling has been frequently used to modify surfaces with amino acids and peptides [11]. The next two amino acids — glycine and aspartic acid — would react respectively to form a proper RGD sequence.

IR spectra of surfaces were obtained with Fourier Transform Infrared Attenuated Total Reflectance (FTIR--ATR). The amount of grafting in PU samples was determined by the colorimetric method with Toluidine Blue O [12]. The poly(acrylic acid) content was obtained form a calibration plot of the optical density of the desorbed dye at 633 nm.

Results and discussion

Studies revealed that 6 hours is a the proper time of PU hydroxylation with 30% H_2O_2. Figure 4 presents the FTIR-ATR spectra of unmodified PU surface and PU after hydroxylation with 30% H_2O_2 under UV radiation. Signals characteristic for -OH group (3600–3000 cm^{-1}) indicate hydroxylation of PU surface. When hydroxylation time was shorter, the –OH signal was less intensive. Longer times caused thermal deformation of polyurethane, as UV lamp emits heat. Selected parameters for acrylic acid grafting is 5% (v/v) of acrylic acid and 1,5 hours of reaction. Figure 5 shows a graph of concentration of –COOH groups on PU surface per 1 cm^2 depending on reaction time for 5% (v/v) of acrylic acid and different temperatures (25, 35, 40 and 45°C). The biggest number of carboxyl groups was grafted in 35°C. For comparison, an analogues graph for 20% (v/v) of acrylic acid was presented. Higher than 5% concentration of acrylic acid, higher temperature and longer reaction time caused homopolymerization of acrylic acid and decreased yield of grafting. Also, acrylic acid homopolymerization led to thick formation of unstable and easy to rub off poly(acrylic acid) layer.

Conclusions

It was proved that carboxyl groups have been successfully introduced on the polyurethane surface. Suitable parameters for acrylic acid grafting were selected. The process of peptides grafting onto so-prepared polyurethane would be elaborated with the use of carbodiimide method. To indicate the presence of amino acids XPS spectra would be obtained. Final materials would be used for endothelial cells culture.

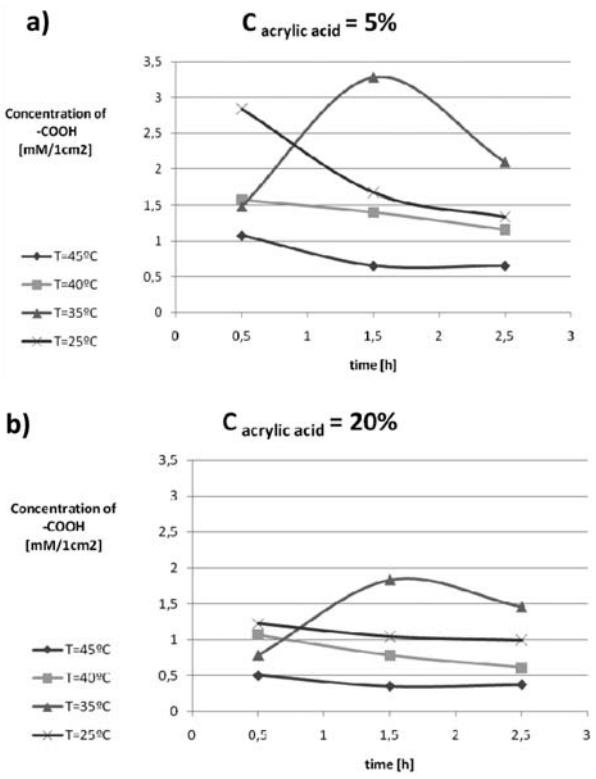

Fig. 5. Carboxyl groups on PU surface depending on reaction time for a) 5% and b) 20% (v/v) acrylic acid at different temperatures.

Acknowledgements

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