Functionalization of polyurethane surfaces for further attachment of bioactive molecules

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The challenge for cardiovascular tissue engineers is to design hemocompatible biomaterials that promote neo-tissue formation. Cardiovascular implants are prone to occlusion caused by surface thrombogenicity. In native tissue non-thrombogenic surface is provided by the endothelium. Endothelialization of implantable cardiovascular devices is thereby among the techniques of functionalizing biomaterials. Surfaces covered with peptides have been shown to enhance endothelial cells adhesion and proliferation. For the purpose of further cell-specific peptides immobilization, a three-step method for incorporating carboxyl groups onto a polyurethane surface was developed. In the first step silanol groups were incorporated into the polyurethane surface. Successful reaction was proven by FTIR analysis. Subsequently, incorporation of surface amine groups was proceeded. In the last step amine groups were acylated using glutaric anhydride to create carboxylates. To determine the presence of surface functional groups, colorimetric method was applied. Measurement of water contact angle revealed significant increase in surface hydrophilicity.

Keywords: material engineering, surface modification, polyurethane modification, polyurethane activation, silanization.

Introduction

Despite constant progress in clinical technologies, there is a great concern over long-term performance of current devices used in cardiosurgery. Low patency rates of vein grafts, thrombosis and calcification of implants have spurred scientists on to develop new strategies. Tissue engineering is an interdisciplinary field that involves the interaction of clinical medicine, engineering and science. The goal of this discipline is to design living tissues, which can replace or regenerate organs with disorders.

In order to achieve neotissue formation three-dimensional cell-instructive material is required. The scaffold provides a mechanical support for cells, encourages proliferation and differentiation of cells and serves to maintain structure and function of the newly created tissue. Biocompatibility, non-thrombogenicity and non-inflammability are among the most important requirements for biomaterials [1].

On account of lining the lumen of blood vessels endothelial cells (EC) are a natural choice for vascular graft seeding. EC create a non-thrombogenic surface and prevent occlusion. In vivo structural support for cells is provided by the extracellular matrix (ECM), composed of proteins, glycoproteins and proteoglycans. Moreover, the ECM contains a wide range of growth factors and cell signalling motifs [2]. These ECM features prompted tissue engineers to develop parallel coating on polymer scaffolds.

Since the introduction of cardiovascular tissue engineering synthetic, natural and bio-inspired materials were applied [3]. These include polyterephthalate (e.g. Dacron®), expanded polytetrafluoroethylene (ePTFE) [4] and polyurethane (PU) [5]. Coating of biomaterials with ECM proteins such as collagen [6] or fibrin [7], just to name a few, and short peptides such as Arg-Gly-Asp (RGD) [8], Tyr-Ile-Gly-Ser-Arg (YIGSR) [9] and Arg-Glu-Asp-Val (REDV) [10, 11] led to enhanced endothelial cells adhesion.

Strong covalent bond via peptides and biomaterial is required for ample cell adhesion [8]. Most of commercially available polymers have inert nature and surface activation prior to biomolecule immobilization is essential. If generated active groups aren’t of desired type or quantity, there is need for the second step — introduction of reactive functional groups specific for
desired biomolecules [12]. Surface modification methods include i.a. ionized gas treatments, flame treatment, UV irradiation, chemical grafting as well as various chemical addition reactions (e.g., acetylation, fluorination, silanization, incorporation of sulfonate groups) and functional group modifications (e.g., oxidation, reduction) [12–14].

The aim of presented study was to activate polyurethane surface by functional group introduction. Modification techniques and surface characterization after each stage are detailed. In the first step reaction of PU functional groups and silicon tetrachloride is proceeded. After hydrolysis, (3-aminopropyl)triethoxysilane chains with amine functional groups are incorporated. In the last step, carboxylated surface is achieved by reaction with glutaric anhydride. In the course of further study it is planned to immobilize HUVEC-adhesive peptides onto the carboxylated surfaces and evaluate the possibility of surface endothelialization.

Materials and methods
PU (medical grade ChronoFlex® C45D, CardioTech) and N,N-dimethylacetamide (DMAC, Sigma-Aldrich) were used to prepare PU films. Molecular sieves (3A, Sigma-Aldrich) were used to dry toluene and acetone (Chempur). Silicon tetrachloride (STC), (3-aminopropyl)triethoxysilane (APTES), glutaric anhydride and triethylamine were obtained from Sigma-Aldrich. Ethanol and acetic acid were purchased from Chempur.

The procedure of PU films fabrication was as follows: PU pellets, previously cleaned with alcohol and dried, were dissolved in DMAC (20% w/v) and transferred on glass to form 0,5 mm thick films. Films were dried at RT until the constant weight was reached. Next, 12 mm-diameter-discs were cut from films and washed with 20% ethanol aqueous solution and subsequently with distilled water. In the first step of surface modification PU discs were immersed in STC/anhydrous toluene solution (5% v/v) for 5 min and then washed with toluene to remove excess STC. Afterwards PU discs were left in room temperature for 24 h, allowing hydrolysis to occur.

In the second step 4% ethanol/water (v/v) solution was prepared and pH adjusted to 4,5 with acetic acid. APTES was dissolved in the prepared ethanol/water solution to the concentration of 4% (v/v). PU discs were immersed in the APTS solution for 2 h and afterwards washed with water/ethanol mixture. Modified substrate was incubated at 30°C for 24 h to remove water and form siloxane bonds. In the third step of the modification process surface amine groups were carboxylated using the following protocol. Glutaric anhydride/acetone solution (30% v/v) was prepared and triethylamine (proton acceptor) was added to a concentration of 2 mg/ml. The modified surfaces were dipped in the solution for 2h and then rinsed with water and dried at 30°C.

Polyurethane surface with silanol groups was characterized by ATR-FTIR spectroscopy (Nicolet 6700, Thermo Scientific). Methyl orange (Sigma-Aldrich) was used to determine surface amine groups. The dye can stoichiometrically combine with positively charged amine groups under acidic condition and dissolve in basic solution. Amount of the surface-bonded MO was determined by absorption measurements. To monitor hydrophilicity of the surface CAM 200 Optical Contact-angle Goniometer (KSV Instruments) was used. Water contact angle (CA) was measured with Attension Theta Software (vers. 4.1.0, Biolin Scientific) after each step of the modification process.

Results and Discussion
To support cell adherence moderate surface hydrophilicity is required [15–18]. Hydrophilic surface is also desired as it resists protein adsorption and can potentially prevent induction of thrombus formation [19]. In order to improve PU hydrophilicity and activate inert PU surface, reaction with STC was proceeded, as illustrated in the Fig.1. Fig. 2 shows the IR spectra of unmodified PU and surface with silanol groups. There is a noticeable difference between the IR spectra. Modified surface shows the band at 1060 cm⁻¹ due to silica (Si-O-Si). The band at 3000–3600 cm⁻¹, characteristic for hydroxyl groups, indicates successful PU-STC hydrolysis. Hydroxyl groups introduction was confirmed by water contact angle measurement. Fig. 3A shows unmodified polyurethane with CA 73,5°. After first step of modification PU discs had a CA of 55,7° (Fig. 3B).

Binding biomolecules, such as peptides, directly to the biomaterial surface can result in steric hindrance and loss of bioactivity [20]. To avoid these undesirable effects a “spacer” is required. In this study APTES is used as a spacer molecule (Fig. 4). Peptides are typically linked to spacers via a stable amine bond, which is created by surface carboxyl groups and N-terminus of the peptide. Moderate concentration of carboxyl groups (Fig. 4), which create negatively charged surface, can

![Fig. 1. First step of the surface modification procedure: reaction of urethane group with STC.](image-url)
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Fig. 2. ATR-FTIR spectra of unmodified PU and after PU-STC hydrolysis.

Fig. 3. Water contact angle of (A) unmodified PU, (B) PU-STC after hydrolysis, (C) PU with surface amine groups, (D) carboxylated PU.
also enhance cell growth [21]. Concentration of amine groups on unmodified PU (0.06 mmol/cm²), after reaction with APTES (4.92 mmol/cm²) and after carboxylation (0.71 mmol/cm²) is shown in the Fig. 5. The results prove that amine groups were successfully incorporated to PU surface and then reacted with glutaric anhydride. What is more, amine groups enhance hydrophobicity to 64.5° (Fig. 3C). Reaction with glutaric anhydride makes the surface more hydrophilic (CA 48.2°, Fig. 3D).

Conclusions

In this study a method of PU surface functionalization for further peptide immobilization and endothelial cells adhesion was developed. Carboxylated surface was successfully achieved in a three-step procedure. Further investigation involves activation of obtained carboxylated surfaces using an active esters, e.g. N-hydroxysuccinimide (NHS) ester and coupling with chosen endothelial cell adhesion peptides such as RGD or REDV.

References


