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Chemical surface modification of parylene C for enhanced protein immobilization and cell proliferation

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ABSTRACT

To introduce the adhesion site of proteins and/or cells on parylene C (PC)-coated medical devices that can be used as implantable biosensors or drug delivery capsules, the PC surfaces were initially modified by the Friedel–Crafts acylation reaction to generate active chlorines. These chlorines were then employed to initiate the atom transfer radical polymerization of *tert*-butyl acrylate (TBA) and form a polymer brush layer of polyTBA on PC; the acrylate groups in the polymer brushes were hydrolyzed to carboxylic acid groups and further activated into succinimidyl ester groups via the 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide/N-hydroxysuccinimide coupling reaction. The PC surface grafted with polymer brushes and activated by succinimide showed efficient attachment of proteins, including gelatin, contortrostatin (CN) and bovine serum albumin (BSA), all at high density on the PC surface. The CN density on the surface was evaluated for both monolayer and polymer brush-based coatings. Based on fluorescence measurements, the polymer brush gives a 60-fold higher surface protein density than the monolayer-based system. Gelatin was used as a model protein and covalently coated onto the modified PC surface for cell culture study. Substrates with gelatin coating showed a significantly higher cell attachment and proliferation in 7 days cultures as compared to the uncoated substrates. In addition, a conventional photolithography technique was coupled with the surface chemistry to successfully pattern the BSA labeled with fluorescein isothiocyanate on the modified PC surfaces.

1. Introduction

Poly(2-chlor-para-xylylene), also named parylene C (PC), has been used as the hermetic packaging material for various long-term implantable electronic devices, such as cardiac pacemakers, neural prostheses, deep brain stimulators, retina tissue prostheses and other biosensors [1–4]. PC can be coated onto substrates to form a conformal, pinhole-free, dense, chemically inert thin layer by a chemical vapor deposition (CVD) process without the use of any solvents [5]. This layer exhibits good biocompatibility (FDA USP Class VI), high dielectric strength, very low permeability against water or ions, and non-degradation in the human body. The medical devices encapsulated by PC can remain stable for many years once implanted [6,7].

Despite the advantages of its use on implantable electronic devices, PC has long been recognized as having the disadvantage of poor protein, cell and tissue adhesion, which limits its application for some implantable biomedical devices. For example, the coating materials for neural devices, such as electrode arrays in the central and peripheral nervous systems, must be able to promote cell adhesion and proliferation for stable neural signal recording and transmitting over many years of implantation [2]. Pristine PC is not suitable as a hermetic material for these electronic devices, which need sustained adhesion to the tissue. To solve this problem, various physical and chemical treatments on the PC surface have been employed to improve its cell adhesion ability; these methods include surface roughening, sculpturing, chemical modification of para-xylylene, ion beam assisted deposition, plasma or electron beam bombardment, and ultraviolet (UV) light irradiation [3,8–11]. Enhanced cytocompatiblity, which is a sign of improved biocompatibility, has been observed on the modified PC surfaces in some of these studies. Most of the PC surface modification processes were designed to either increase the surface roughness or reduce the surface hydrophobicity, so as to improve the non-specific mammalian cell attachment and spreading.

Protein immobilization on the PC surface has been reported. For example, Chang et al. [12] coated PC films with fibronectin by a physical coating process. The fibronectin-attached PC surface exhibited the same hydrophobicity as the untreated PC surface, but had dramatically higher fibroblast and hepatocyte attachment and proliferation, indicating that cell adhesion mechanisms vary on surfaces with similar hydrophilicity but different chemistries

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[13]. By immobilization of PC with selected proteins, the PC surface may obtain versatile biological functionalities, including strong support for the desired cell attachment, prevention of unwanted cell attachment, improvement of biocompatibility and enhancement of tissue adhesion. Compared with the physical coating process previously reported, the covalent binding process via chemical reactions provides a significantly higher protein-substrate affinity, as well as a stable biological activity of the modified PC substrate. This will be of great interest for long-term medical devices implanted in a harsh tissue environment. The present paper involves a covalent approach for high-density protein attachment to PC.

The generation of patterned protein and cell surface coatings on implanted medical devices has been studied for tissue engineering applications [14–16]. The immobilization of various types of proteins, pharmaceutical drugs and biologically active molecules at designated places on the substrate may further enhance the functionalities of medical devices. For example, the surface of medical devices can be modified to be tissue adhesive, metal adhesive and drug deliverable [17,18]. Photolithograpic techniques, initially developed in the semiconductor industry, have been used in the biomedical diagnostics field, and are the most frequently applied techniques for patterning functional molecules on planar substrates [19–23]. However, despite the wide use of PC in the medical field, reports of chemical modification of the PC surface is limited, and there has no been related research reported on the patterning of the PC surface for biomedical applications.

In present work, we applied a series of chemical modification reactions to give PC substrates with a high density of bioconjugating groups (succinimidyl esters) on the PC surface. This material gives a high level of protein coverage on subsequent treatment with aqueous solutions of the chosen protein. To understand the feasibility of the protein patterning process, we have further applied the traditional photolithography technique to pattern bovine serum albumin (BSA) that is labeled with fluorescein isothiocyanate (FITC) onto a PC substrate. The PC surface chemical characteristics, morphology, cytocompatibility and protein patterning technique were analyzed, evaluated and addressed.

2. Materials and methods

2.1. Materials

Parylene-C (100 μm in thickness) films were prepared on glass slides by the CVD technique using PDS2010 Labcoater (Specialty Coating System Company, Indianapolis, IN). The PC films were peeled from the glass slides, soaked in dimethylformamide (DMF) and acetone for 30 min each, to ensure removal of the unreacted dimer [24], then vacuum dried at room temperature. The cleaned PC film was cut into squares (1.5 × 1.5 cm) before surface chemical modification.

Tert-butyl acrylate (TBA; Sigma–Aldrich) was passed through a column filled with inhibitor removers (Sigma–Aldrich) to remove the trace amount of hydroquinone monomethyl ether. 2-Chloropropionyl chloride (CPC; Sigma–Aldrich) was distilled to remove impurities. Azobisisobutyronitrile (AIBN; Sigma–Aldrich) was crystallized from methanol before use. Dichloromethane (VWR) was distilled from CaH_2 to remove the remaining moisture. Anhydrous aluminum trichloride (Fluka), succinimide anhydride, copper (I) chloride (Sigma–Aldrich), 1,1,4,7,10,10-hexamethyltriethylenetetramine (HMTETA; Sigma–Aldrich), methanesulfonic acid (Fluka), N-hydroxysuccinimide (NHS; Sigma–Aldrich), N-(3-dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride (EDC; Sigma–Aldrich), 2-(N-morpholino) ethanesulfonic acid hydrate (MES hydrate; Sigma–Aldrich), gelatin from bovine skin (type B, Sigma–Aldrich), BSA fraction V (Sigma–Aldrich), Coomassie Brilliant Blue R250 (Bio-Rad Lab), FITC (Sigma–Aldrich) and AZ1512 photoresist (Clariant Corp.) were used as received without further purification. Contortrostatin (CN), a protein from snake venom, was purified by multi-step high-performance liquid chromatography according to the previous report [25].

2.2. Synthesis of bulk poly(tert-butyl acrylate) (poly-tBA)

Bulk poly-tBA was synthesized by traditional free radical polymerization method in DMF. TBA was mixed with AIBN at a molar ratio of 100:1 to form a 20 wt.% solution; this solution was then heated to 65–70 °C and maintained overnight under nitrogen. The TBA polymer was precipitated into a large amount of distilled water and dried. The resulting polymer was dissolved and precipitated again to remove impurities. The final polymer was dried at 40 °C for 24 h in a vacuum oven, and used as a control to estimate the amount of poly-tBA grafted onto the PC surface.

2.3. Surface modification of PC films with polymer brushes and the immobilization of protein

2.3.1. The Friedel–Crafts acylation reaction of PC to form active chlorines on PC-Cl

$AlCl_3$ (2.50 g, 18.8 mmol) and CPC (1.79 ml, 18.8 mmol) were mixed in 75 ml of CH_2Cl_2 under nitrogen to form a homogeneous, slightly brown colored solution at 0 °C. PC films were then submerged in this solution and allowed to react at 0 °C for an initial 6 h, followed by 10 h of reaction at room temperature. The films became dark brown over the course of the reaction. After acylation, the films were soaked in DMF for 1 h and then acetone for 1 h until the film became light yellow, after which it was dried in vacuo. The resulting film is designated as PC-Cl.

2.3.2. Surface polymerization of TBA polymer brushes on PC to form PC-TBA

TBA (10 ml) was mixed with 68 mg of CuCl and 187 μl of HMTETA (molar ratio of TBA:CuCl:HMTETA = 100:1:1) to form a green mixture at room temperature under nitrogen. PC-Cl films were immersed in the mixture and heated to 70–80 °C for 4–6 h with gentle stirring to allow the growth of poly-tBA polymer brushes on the surface via an atom transfer radical polymerization (ATRP) reaction. After that, the films were rinsed with acetone, followed by soaking in a 30 vol.% acetic acid/water solution at room temperature for 4 h to remove the attached copper ions. The films were then washed with acetone and vacuum dried at room temperature. These films grafted with poly-tBA brushes had a light yellow color, and are designated as PC-tBA.

2.3.3. Hydrolysis of ester groups on PC-tBA into acrylic acid groups to form PC-AA

Methanesulfonic acid (4 ml) was added to 20 ml of CH_2Cl_2 to form a 20 vol.% clear mixture, PC-tBA films were immersed in the acidic solution at room temperature and allowed to react for 1–2 h with gentle agitation [26]. The butylacrylate groups on the polymer brushes of PC-tBA were hydrolyzed to give carboxylic acid groups, forming poly(acrylic acid) (PAA) polymer brushes on the substrate. The resulting films were rinsed with a large amount of CH_2Cl_2 and acetone, vacuum dried at room temperature and designated as PC-AA.

2.3.4. Activation of carboxyl groups on the polymer brushes of PC-AA into succinimidyl ester groups to form PC-SE

EDC (80 mg, 0.42 mmol) and NHS (48 mg, 0.42 mmol) were dissolved in 10 ml of 0.05 M MES buffer (pH ≈ 5.0) at room temperature to form a clear solution under nitrogen. PC-AA was added to the solution and soaked at room temperature with gentle stirring