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Full Length Article

Surface conjugation of poly (dimethyl siloxane) with itaconic acid-based materials for antibacterial effects

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ABSTRACT

Poly (dimethyl siloxane) (PDMS) is widely used in various biomedical applications. However, the PDMS surface is known to cause bacterial adhesion and protein absorption issues due to its high hydrophobicity. Therefore, the development of antibacterial and anti-protein products is necessary to prevent these problems. In this study, to improve its antibacterial property and prevent protein adsorption, PDMS surfaces were conjugated with itaconic acid (IA) and poly (itaconic acid) (PIA) via a chemical method. Additionally, IA and PIA were physically blended with PDMS to compare the antibacterial properties of these materials with those of the chemically conjugated PDMS surfaces. The successful synthesis of the PIA polymer structure was confirmed by proton nuclear magnetic resonance (^{1}H NMR) spectroscopy. The successful conjugation of IA and PIA on PDMS was confirmed by attenuated total reflection-Fourier transform infrared spectroscopy (ATR-FTIR), X-ray photoelectron spectroscopy (XPS), scanning electron microscopy (SEM), water contact angle measurements, and microbicinchoninic acid (BCA) protein assay analyses. The PDMS surfaces functionalized with IA and PIA by the conjugation method better prevented protein adsorption than the bare PDMS. Therefore, these surface-conjugated PDMS can be used in various biomedical applications.

1. Introduction

Poly (dimethyl siloxane) (PDMS) is widely used as a biomaterial because it is non-toxic, inert, optically transparent, non-flammable, permeable to gasses, and has excellent mechanical properties [1–3]. Although PDMS has many advantages, the PDMS surface is known to cause bacterial adhesion and protein absorption issues due to its high hydrophobicity [4,5]. Generally, antibacterial agents have been used to prevent bacterial film formation on PDMS surfaces [6,7]. However, the emergence of antibiotic-resistant bacteria is becoming an increasingly serious health problem with the ineffectiveness of antibacterial drugs [8].

Recently, there have been other studies on treating the surface of PDMS [9–11]. Also, Komaromy et al. investigated the effect of changing the physical properties by increasing the hydrophilicity by UV radiation; the PDMS surfaces showed a promising repellent effect against both live and dead *Escherichia coli* and *Staphylococcus aureus* cells [12]. Although these methods proved to be effective, challenges remain, including the reduced biocompatibility found after chemical treatment, hydrophobicity recovery, and physical damage to the PDMS surface [13,14]. Thus, the design and modification of PDMS surfaces using biomaterials that inhibit bacterial growth has been investigated to improve current medical devices [15]. Wu et al. demonstrated a method for producing chlorogenic acid (CA)-modified PDMS substrates to increase the antimicrobial properties and improve the viability of the target cells [16]. More recently, Xu et al. reported the prevention of bovine serum albumin (BSA) adsorption and reduction of bacterial adhesion with (mucin/poly (ethyleneimine)) $_n$-functionalized PDMS films [17]. Hou et al. grafted poly(ethylene oxide) onto thermoplastic (carboxylated cardopoly(aryl ether ketone), PEK-COOH) membrane surfaces via the carbodiimide/*N*-hydroxysuccinimide EDC/NHS methodology to modify the membrane hydrophilicity, and excellent renewable antimicrobial and antifouling activities were found [18]. Wang et al. synthesized a novel poly (methacrylisobutyl polyhedral oligomeric silsesquioxane-*co*-2-(dimethylamino)-ethyl methacrylate) (p (MA POSS-*co*-DMAEMA)) coating by using a reversible addition–fragmentation chain-transfer (RAFT) polymerization method to improve the antibacterial and anti-adhesive properties of PDMS membranes. After being quaternized by treatment with 1-bromoheptane, the resulting p (MA POSS-*co*-DMAEMA$^+$) brushes showed a remarkable bactericidal activity against *S. aureus* owing to the p (DMAEMA$^+$) component [19].

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Itaconic acid (IA) is a naturally occurring non-amino and non-toxic organic acid that shows a good antimicrobial activity [20–22]. Recently, Sood et al. reported the synthesis of an IA-conjugated carboxymethyl cellulose-cl-poly (lactic acid-*co*-itaconic acid) hydrogel via a facile graft copolymerization using *N*-*N*[1]-methylene-bis-acrylamide (MBA) and potassium persulfate as the crosslinker and initiator. The prepared hydrogel showed excellent antimicrobial activities against *S. aureus* and *E. coli* [23]. Krezovic et al. reported a series of semi-interpenetrating polymer networks (semi-IPNs) of 2-hydroxyethyl acrylate and IA hydrogels, in the presence of poly (*N*-vinylpyrrolidone) via free radical copolymerization. The synthesized hydrogels showed the best antibacterial activity against *Pseudomonas aeruginosa*. From this study, it is apparent that the IA content and time of exposure greatly influence the antibacterial potential [24]. Therefore, in this study, we chemically and physically conjugated IA and poly (itaconic acid) (PIA) to the surface of PDMS to investigate their antibacterial properties. Herein, we have prepared substrates including IA- and PIA-conjugated PDMS, IA- and PIA-blended PDMS, and conjugated and blended IA- and PIA-PDMS using both a chemical conjugation and physical blending method to enhance the bactericidal efficacy.

2. Experimental

2.1. Materials

The PDMS elastomer (Sylgard® 184 silicone elastomer kit) was purchased from Dow Corning, USA. Itaconic acid (IA, analytical grade, assay ≥99%, M_W: 130.10 g/mol, density: 1.573 g/mL at 25 °C (lit.)), choline chloride (ChCl, assay ≥ 99%, M_W: 139.62 g/mol), ammonium persulfate (APS, assay ≥ 98%, M_W: 228.20 g/mol), *N*-hydroxysuccinimide (NHS, assay ≥ 98%, M_W: 115.09 g/mol), (3-aminopropyl)triethoxysilane (APTES, assay ≥ 99%, M_W: 221.37 g/mol, density: 0.946 g/mL at 25 °C(lit.)), and bovine serum albumin (BSA, purified by heat shock fractionation, pH 7, assay ≥ 98%, M_W: ~66 kDa) were purchased from Sigma-Aldrich, South Korea. Dulbecco's Modified Eagle's Medium (DMEM), Dulbecco's phosphate buffered saline (DPBS/Modified 1X, pH 7.4), trypsin 0.25% (1X) solution, and antibiotic/antimycotic solution (100X) were purchased from GE Healthcare Life Sciences HyClone Laboratories, USA. NIH 3T3 mouse fibroblast cells (NIH 3T3) were purchased from Korean Cell Line Bank (KCLB). Rhodamine-Phalloidin/DAPI (4′,6-Diamidino-2-Phenylindole, Dihydrochloride were purchased from (Invitrogen, Thermo Fisher Scientific, USA). 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide HCl (EDC.HCl, assay ≥ 99%, M_W: 191.70 g/mol) was purchased from Carbosynth Limited, UK. The Micro BCA™ protein assay kit was purchased from Thermo Scientific, USA. Cellulose membranes (molecular weight cut off (MWCO): 3500 Da) were purchased from Spectrum Laboratories, Inc., Canada. Distilled water (DW) was used for the preparation of solutions and washing. *E. coli* DH5α (KACC13821), *Bacillus subtilis* (KACC10111, ATCC465), and *S. epidermidis* (KCCM21205, ATCC12228) were obtained from the Korean Agricultural Culture Collection (KACC). The culture medium was commercial. The LIVE/DEAD BacLight bacterial viability kit was purchased from Thermo Fisher Scientific, USA. All chemicals were used as received without further purification.

2.2. Synthesis of poly (itaconic acid) (PIA)

PIA has been previously prepared by free radical polymerization [25]. A closed 100 mL glass bottle containing 25 mL of freshly distilled water, 5.20 g IA (40 mmol), 0.91 g choline chloride (4 mmol), and 0.456 g ammonium persulfate (2 mmol) was held at 55 °C for 96 h (on a stirring hotplate at 130–135 °C) with constant stirring at 300 RPM. Next, the solution was dialyzed for 1 week through a cellulose membrane (MWCO: 3500 Da) against distilled water to remove the low-molecular-weight products, and then finally lyophilized for 96 h. The molecular weight and molecular weight distributions of PIA were assessed by gel permeation chromatography (GPC, Agilent 1100 S, Germany). This used a linear column, with aqueous eluents (0.1 M sodium nitrate/0.01 M sodium dihydrogen phosphate (pH 7)) at a flow rate of 1 mL/min, and a refractive index detector (RID A). Polyethylene glycol standards were used for calibration.

2.3. Preparation of IA- and PIA-conjugated PDMS surfaces

2.3.1. Preparation of PDMS

The PDMS precursor solution (Sylgard® 184 silicone elastomer kit) is composed of a base (part A) and curing agent (part B). The base and curing agent (10:1 by mass) were fully mixed; then the mixture was evenly spread on a Petri dish, degassed in a vacuum desiccator for 5 min to remove any air bubbles, and cured in an oven at 70 °C for 60 min. After being sectioned into 1 mm-thick spherical disks with a diameter of 1.2 cm, the PDMS surfaces were washed with 70% ethanol and dried in a fume hood for 12 h.

2.3.2. Surface conjugation of PDMS with IA monomers

To prepare the IA- and PIA-conjugated PDMS surfaces, the cleaned PDMS surfaces were treated with O_2 plasma for 1 min (CUTE-1B, Femto Science, USA) and subsequently immersed in 5 wt% APTES (in DW) at 60 °C for 2 h with constant stirring (200 RPM). Following the APTES treatment, the PDMS surfaces were thoroughly washed with 70% ethanol to remove any unreacted APTES molecules. IA solutions were prepared separately in DW at concentrations of 50 and 150 mmol with the appropriate amounts of EDC (10 and 50 mmol) and NHS (10 and 50 mmol). The PDMS surfaces were incubated in an IA reaction mixture for 2 h at 60 °C (on a stirring hotplate at 135–140 °C) with constant stirring at 200 RPM. Unreacted IA and EDC/NHS residues were thoroughly washed away with 70% ethanol. The IA-conjugated PDMS surfaces were dried in a fume hood at room temperature (RT) for 12 h.

2.3.3. Surface conjugation of PDMS with PIA polymer

The O_2 plasma and APTES treatments given in detail in Section 2.3.2 were performed. PIA solutions were prepared separately in DW at concentrations of 0.25 and 0.50 wt% of the appropriate amounts of EDC (10 mmol) and NHS (10 mmol). APTES-treated PDMS surfaces were incubated in the PIA reaction mixture for 2 h at 60 °C (on a stirring hotplate at 135–140 °C) with constant stirring at 200 RPM. Unreacted PIA and EDC/NHS residues were thoroughly removed with 70% ethanol. The PIA-conjugated PDMS surfaces were dried in a fume hood at RT for 12 h.

2.3.4. Physical blending of IA and PIA with PDMS

First, the four solutions (IA 500 mmol, IA 2000 mmol, PIA 2 wt%, and PIA 8 wt%) were prepared separately in DW. Each solution was separately mixed with the PDMS precursor base (part A) and curing agent (part B) at a ratio of 10:1 by mass. Each mixture was evenly spread on a Petri dish (14.5 cm diameter), degassed in a vacuum desiccator for 5 min to remove any air bubbles, and cured in an oven at 70 °C for 60 min. Spherical disks with a diameter of 1.2 cm and a thickness of 1 mm were used for the following studies.

2.3.5. Surface conjugation of IA- or PIA-blended PDMS surfaces with IA and PIA

To prepare the IA-conjugated blended IA/PDMS surfaces, the cleaned blended 500 and 2000 mmol IA/PDMS surfaces were treated with O_2 plasma then immersed in APTES (see Section 2.3.2). Subsequently, the PDMS surfaces were thoroughly washed with