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Improvement of biofouling resistance on bacterial cellulose membranes

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ABSTRACT

Bacterial cellulose possesses excellent biocompatibility and mechanical strength that show great potentials for biomaterial applications. In this study, the surface modifications of bacterial cellulose (BC) membranes were facilitated using either simple coating or chemical grafting methods. The surface coating method is to simply immobilize BC membranes with poly(ethylene glycol) (PEG) solutions of concentration from 1 to 10%, followed by post-treatment with argon (Ar) plasma. The chemical method involved grafting mPEG (monofunctional methyl ether PEG) on BCs. The outcomes of surface modifications were characterized by surface chemical compositions (electron spectroscopy for chemical analysis (ESCA), Fourier transform infrared (FTIR) spectroscopy, X-ray diffraction (XRD), thermogravimetry analysis (TGA), and surface morphology (atomic force microscopy (AFM) and scanning electron microscopy (SEM)). The effects of resistance to biofouling were verified by quantifying the adsorption of proteins and mammalian cells. The results showed that the PEG coating on BCs improved the resistance to cell adhesion by more than 30%. On the other hand, the specific chemical grafting resulted in a particularly high resistance to biofouling that the density of adherent cells reduced by more than 70% when compared to that on pristine BC. We have demonstrated that the two proposed methods were effective for the preparation of bioinert BC membranes with great potentials for applications in biomaterials and tissue engineering.

1. Introduction

Microbial cellulose, a natural hydrogel-like polymer, is synthesized by the acetic bacterium *Acetobacter xylinum* in either static or agitated culture conditions. The high specific surface area, elasticity, wet strength and conformability characteristics of the bacterial cellulose fibrils are of great research interests for applications in food and beverage processing and in industries for the production of paper, plastic, membranes for separation, and fuel cells [1–6].

Microbial cellulose is also a good candidate for biomaterials due to its naturally high water content, high temperature resistance, good mechanical properties and biocompatibility. In particular, the non-toxicity and non-mutagenicity enable microbial cellulose to be a popular candidate for wound healing dressings and tissue engineering scaffolds [7–11]. For example, Klemm et al. reported using bacterial cellulose for temporary substitutes for skin and artificial blood vessels [12]. The tubular BASYC® bacterial cellulose was fabricated as an artificial blood vessel and showed good mechanical strength in a wet state which exhibited high water absorption ability and low inner surface roughness [12]. In addition, Zaborowska et al. have altered the porosity of BC membranes for the support of bone regeneration [13].

For biomedical implants, one of the most important considerations is the surface fouling phenomena that occur due to the nonspecific adsorption of proteins and undesired biomolecules [14–16]. The strategies that have been developed to reduce biofouling associated to graft the surface of materials with antifouling molecules or to incorporate self-assembling monolayers (SAMs) on the surface of materials [17,18]. The most commonly used antifouling molecules are polyacrylates [19,20], oligosaccharides and phospholipids [21–23], and poly(ethylene glycol) [24–26]. Among all the antifouling moieties, PEG is the most studied one due to its chain mobility, excluded volume effect, and osmotic repulsion which are responsible for the formation of a protective layer on the underlying surfaces [27–29]. Moreover, PEG is recognized as biocompatible and weakly immunogenic due to its weakly basic ether linkage that resists the adsorption of proteins [30]. The reported methods for incorporating PEG onto materials were generally facilitated by either physical or chemical methods.

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The preparation of fouling resistant surfaces via PEG immobilization is the simplest method, although immobilization method is subjected to the limitations such as chemical specificity of the substrates, lengthy synthetic procedures, and thermal and hydrolytic instabilities [31,32]. The BC/PEO (poly(ethylene oxide)) and BC/PEG composites or nanocomposites were prepared by adding PEO or PEG to the growth medium of *A. xylinum* [33]. Moreover, the immobilization of BC membranes with different molecules such as PEG, chitosan, gelatin, polyvinyl alcohol (PVA), or hydroxyapatite (HAp) was reported to have improved biocompatibility when compared with the pure BC and therefore showed versatile applications in wound dressing materials, cardiovascular tissues, and artificial bones [34–37].

It is also possible to incorporate fouling resistance by chemical methods which aimed to incorporate more specific functionalities with stable linkages and better durability. Liu and coworkers have grafted zwitterionic polymers to cellulose membranes using the atom transfer radical polymerization (ATRP) method that the resistance to the adhesion of nonspecific proteins and platelets was significantly improved [38]. In addition, Nge and Sugiyama modified BC microfibrils using 2,2,6,6-tetramethylpyperidine-1-oxyl (TEMPO) mediated oxidation to incorporate active carboxyl functional groups and to promote the rate of apatite nucleation [39]. However, the chemical grafting of PEG on bacterial cellulose was rarely reported, which is probably due to the complexity and the low reactivity of the hydroxyl groups of BC. It is therefore desirable to use more reactive compounds or to increase the surface reactivity for the surface modification on BC [40,41]. In this study, two methods were proposed to incorporate PEG on BCs. Firstly, BC membranes were simply immersed in PEG solutions and followed by Ar plasma post-treatment to form PEG coated BC (PEG-*c*-BC). Ar plasma was employed for surface modification for the improvement of surface hydrophilicity and to assist surface crosslinking [42].

Ar plasma assists the generation of free radicals to crosslink PEG on BCs [43]. The second method involved chemically grafting monofunctional methyl ether PEG (mPEG) onto BCs using two reaction steps. mPEG is used as a starting molecule to react with isophorone diisocyanate (IDPI) for the incorporation of highly reactive isocyanate groups on mPEG, forming mPEG-IPDI. The second step is to add excess IPDI with mPEG (IPDI:mPEG = 10:1, in molar ratio) that mPEG reacted predominantly with the more reactive alicyclic group (CH_2NCO, rather than the aliphatic moiety, CHNCO) of IDPI such that the formation of the byproduct, block polymer mPEG-IPDI-mPEG, was avoided [44,45]. The sole isocyanate group on mPEG-IPDI then further reacted with the hydroxyl groups on BCs to form urethane linkages, resulting in mPEG-*g*-BC [43,44]. The fouling resistance was examined by directly immobilizing proteins or by cultivating mammalian cells on the prepared BC membranes. For the potential developments of BCs for wound dressing and skin replacements, L-929 fibroblasts were chosen for evaluating the interactions with BCs. The results showed that the fouling resistance was improved by more than 1.3-fold on the PEG-*c*-BC membranes. On the other hand, BC membranes that contained the chemically grafted mPEG revealed more than 1.7-folds antifouling efficacy over the native BC. This study provides effective routes for the surface modifications on bacterial cellulose membranes that the high biofouling resistance is promising for further applications in wound dressings and tissue engineering scaffolds.

2. Materials and methods

2.1. Materials

All reagents and chemicals were purchased from Sigma (USA) unless otherwise specified. The bacterial cellulose utilized in this study was *Gluconacetobacter xylinum* (BCRC12335), kindly provided by FIRDI (Food Industry Research and Development Institute, Hsinchu, Taiwan), which was cultured in 500 ml of mannitol broth (MB) medium containing 25 g/L mannitol, 5 g/L yeast extract and 3 g/L peptone in a 1 L flask with shaking at 180 rpm for 24 h at 30 °C [46,47]. MB media was inoculated with *G. xylinum* culture (10%, v/v) and cultivated at 30 °C for 14 days statically. Cellulose cake floating on the culture was collected and washed with distilled water for 12 h to remove impurities and followed by treating with NaOH for 10 min, followed by immersion in 0.5 N NaOH for 24 h at room temperature and then rinsing with deionized water to a neutral pH. Wet BC membranes were cultivated from the acetic bacterium *A. xylinum* and were dried at 60 °C overnight, followed by cleaning with 95% ethanol and DI water, prior to each experiment. The sterilization of BC membranes were facilitated by exposing under ultraviolet light for 30 min and sequentially immersed in 95% ethanol and DI water for 15 min.

2.2. Preparation of PEG-g-BC

The physical incorporation of PEG on BC to form PEG-g-BC was performed by immersing BC membranes (7 cm × 3.5 cm) in different concentrations of PEG (M.W. 2000), ranging from 1 to 10 wt%, in DI water overnight. The dried membrane was then treated with Ar plasma under an applied power of 15 W at a flow rate of 20 sccm for 1 min, with a fixed pressure of 100 mTorr, which was followed by rinsing with a copious amount of distilled water to remove the residue of PEG and then drying under reduced pressure to remove all the remaining water and form PEG-g-BC. The prepared membranes were weighted to calculate the grafting density of the poly(ethylene glycol).

2.3. Synthesis of mPEG-BC

The preparation of the chemically grafted mPEG-BC involved the synthesis of mPEG-IPDI and the coupling of mPEG-IPDI with the BC membranes. A schematic presentation for the derivatization reactions is shown in Scheme 1. mPEG-IPDI was first synthesized by distilling IPDI at 120 °C to remove the unbound IPDI which possesses a higher boiling point. Then, excess amount of IPDI was mixed with monofunctional methyl ether PEG (mPEG) according to the procedures proposed by Zhang and coworkers [44]. In brief, 10 g of IPDI, 5 g of mPEG, and 0.09 g of dibutyltin dilaurate (DBTL) were added to 50 ml dried chloroform. The mixture was heated to 70 °C for 15 h under nitrogen flow. After the completion of the reactions, the copolymer was precipitated in petroleum ether, chloroform, and petroleum ether sequentially; this procedure was repeated several times to remove the residue of IPDI from the copolymer, which was then filtered to obtain mPEG-IDPI powder. The coupling of BC with mPEG-IDPI was facilitated by adding 50 ml of chloroform and 0.6 wt% of DBTL to mix with BC membranes and mPEG-IPDI, followed by heating at 70 °C for 24 h to form mPEG-BC.

2.4. Characterizations of the synthesized molecules and the prepared BC membranes

Nuclear magnetic resonance (^{1}H NMR) was performed with d-chloroform ($CDCl_3$) as the solvent (Avance 500 MHz FT-NMR, Bruker). The elemental analyses were performed using MestRe-C® software. The number average molecular weight (M_n), the weight average molecular weight (M_w), and the polydispersity index were determined using gel permeation chromatography (GPC) (system: Waters, 2410 refractive index detector; columns: Styragel HR 4 and HR 2). Tetrahydrofuran (THF) was used as the elution solvent, and the calibration curve was generated using polystyrene standards. For the GPC analyses, 15 mg/ml of sample solution was