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UV-curable enzymatic antibacterial waterborne polyurethane coating

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ABSTRACT

Novel ultraviolet (UV)-curable enzymatic antibacterial coatings were designed and fabricated. The coatings were prepared by simply mixture of castor oil-based cationic waterborne polyurethane-acrylate dispersion (WPUA) and acryloyl chloride modified lysozyme followed by UV curing. The covalent incorporation of lysozyme into the coatings was proved by enzyme leakage and IR analysis of the coatings. As the lysozyme content increased from 0 to 2.5% (w/w), the tensile strength of the coating enhanced by 37% (1.1 MPa *vs* 0.8 MPa), and the contact angle increased by 10% (73.1° *vs* 80.6°). Those changes are possibly due to enhancement of crosslinking degree and hydrogen bonding induced by modified lysozyme. Based on live/dead staining and colonies number assay, the coatings containing lysozyme (2.5%, w/w) were highly efficient in killing *Staphylococcus aureus* (91.3%) and *Escherichia coil* (62.9%) via degradation of cell wall within 2 h, and the colonies number for both strains was significantly decreased to less than 1% after incubation for 24 h. In addition, the killing efficiency of the coatings remained 68.3% after six continuous uses. Such UV-curable waterborne polyurethane coatings containing lysozyme (UWPU-L) may be useful in anti-bacterial stains on various common surfaces in hospital settings and food packaging.

1. Introduction

Bacteria can attach to surfaces of synthetic materials within hours and form a protective extracellular biofilm, resulting in bacterial contamination, which is harmful for medical implants, food packaging, water handling, and shipping industry, etc. [1–5]. Antibacterial coatings have been extensively explored to prevent bacterial attachment or kill attached bacteria. Generally, antibacterial strategies are based on surface modification, such as attachment of polyethylene glycol (PEG), poly (*N*-isopropylacrylamide) (PNIPAAM) to a surface, as well as the embedment of bactericide in the coatings, including silver nanoparticles, antibiotic and quaternary ammonium compounds [6–10]. However, PEG-modified surface it is not as effective in reducing bacterial colonization as in resistance to protein and PEG undergoes oxidation in long term use [11,12]. The extensive use of silver and antibiotic can lead to emergence of silver and antibiotic-resistant strains [13]. Furthermore, the use of biocides may cause side-effects such as cytotoxicity, hypersensibility and inflammatory responses. Replacement of biocides with non-toxic antifouling enzyme has been proposed as a viable strategy [14–17]. For example, lysozyme can damage bacterial cell walls by hydrolysis of peptidoglycans, increasing the bacteria's permeability and causing the bacteria to burst [18]. Proteases and glycosidases can inhibit bacterial attachment by hydrolysis of proteins and polysaccharides which constitute biofilm in bacterial attachment [19].

Antibacterial enzymes have already been immobilized on the surface of materials based on embedding with film-forming polymers or covalent graft on surfaces of materials. For the first way, lysozyme was usually physically mixed with polymers to form coatings [20]. The long term use of the coatings is limited, due to easily release of lysozymes from the coatings. Therefore, some form of immobilization is suggested to counter leaching [19,21]. Covalent immobilization of lysozyme on polymer material surfaces was effective in reducing bacterial adhesion and biofilm formation [22], but the process of immobilization is time consuming and complicated. Incorporation carbon nanotube-enzyme conjugates into polymer coatings provides an effective method to retain high activity and stability of enzymes [23]. However, it has a potential security issue for the toxicity of carbon nanotubes. Covalent embedding of enzymes ensures retention of protein in the support matrix as well as maximizing the advantages of immobilization [24]. Polymerization of modified enzyme with vinyl monomer was shown to be a feasible way to realize covalent embedment of enzymes [25,26]. Unfortunately, most polymer syntheses occurred in organic conditions that would irreversibly denature a part of enzymes. Veg-

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etable oil-based waterborne polyurethanes have been regarded as green materials with highly tunable properties [27]. They show good compatibility to immobilize enzyme and can dramatically improve the stability of enzyme molecules [14].

In this study we prepared UV-curable castor oil-based cationic waterborne polyurethane-acrylate dispersion (WPUA) and double bond modified lysozyme to fabricate enzymatic coatings by UV curing. The polymerization of enzymes with polyurethane-acrylate can lead to covalent entrapment of enzymes and form enzymatic coatings at room temperature. The effects of polymerization of lysozyme on the mechanical, thermal and hydrophilic properties of the UV-curable waterborne polyurethane coating are investigated. The antibacterial capability of the UV-curable waterborne polyurethane coating containing lysozyme (UWPU-L) is characterized by fluorescence and colony analysis.

2. Materials and methods

2.1. Materials

Castor oil was purchased from Alfa Aesar. *N*-methyl diethanolamine (MDEA) was purchased from Ourchem. Acetic acid, dibutyltin dilaurate (DBTL), methyl ethyl ketone (MEK) and 4 A molecular sieves were purchased from Sinopharm Chemical Reagent Co., Ltd. Isophorone diisocyanate (IPDI), 2-Hydroxyethyl methacrylate (HEMA), 2-hydroxy-2-methyl-1-phenyl-1-propanone (Darocur 1173) and acryloyl chloride were purchased from TCI. To remove residual water, castor oil was distilled at 90 °C under vacuum for 2 h and MEK was incubated with 4 A molecular sieves overnight. Lysozyme from chicken egg white and *Micrococcus lysodeikticus* (ML) were purchased from Sigma. *Escherichia coli (E. coil)* and *Staphylococcus aureus subsp. aureus (S. aureus)* were purchased from China General Microbiological Culture Collection Center. LIVE/DEAD BacLight Bacterial Viability Kits were purchased from Invitrogen (Grand Island, NY).

2.2. Preparation of WPUA

A certain amounts of castor oil, IPDI and MDEA were added into a four-necked flask equipped with a mechanical stirrer, nitrogen inlet, thermometer and condenser. After addition of DBLT the reaction was carried out at 60 °C for 3 h and MEK was properly added to reduce the viscosity of the reaction system. Then, the above mixture was reacted with HEMA at 80 °C for another 3 h, and the prepolymer containing carboxyl group was obtained. The molar ratio of the NCO groups of the IPDI, the OH groups of the castor oil, the OH groups of the MDEA and the OH groups of the HEMA was 2.00: 0.49: 0.93:0.57. When the temperature was cooled down to room temperature, acetic acid (1.2 equivalents of MDEA) was added into the flask and reacted for 1 h followed by dispersion with distilled water at a high speed (2000 rpm). After removal of the MEK under vacuum, cationic WPUA with a solid content of 20 wt% was obtained. The synthesis of WPUA was shown in Fig. S1.

2.3. Lysozyme modification

Double bond modification of lysozyme was performed as followed. Lysozyme (50 mg) was firstly dissolved in 10 mL of phosphate buffer (0.1 M, pH 6.2). The enzyme solution was cooled to 4 °C and 20 μL of acryloyl chloride was gradually added to the solution over 10 min with continuous stirring.

2.4. Determination of the extent of chemical modification

The average content of double bond in the modified lysozyme was determined by the TNBSA chromogenic assay [28]. Lysozyme and modified lysozyme (M-lysozyme) were dissolved in PBS buffer (0.1 M, pH 6.2) to achieve concentrations of 0.20, 0.4, 0.6, 0.8, and 1.0 mg mL^{-1}. 0.5 mL of TNBSA (0.01%, w/v) solution and 1 mL of $NaHCO_3$ (4% w/w, pH 8.0) was added to 1 mL of lysozyme and M-lysozyme solution respectively. Then the mixtures were incubated at 40 °C for 2 h. PBS buffer was used as blank. After incubation, 0.5 mL of a SDS (10% w/w) solution and 0.5 mL of HCl (1 M) were added. The absorbance of the solutions at 410 nm was measured with a UV/Vis-spectrophotometer. All absorbance values obtained were corrected with the buffer blanks and plotted versus the enzyme concentration. The percentage of double bond modification was calculated using the equation:

$$\text{Modification rate} = \left(1 - \frac{\text{slope of modificated enzyme}}{\text{slope of free enzyme}}\right) \times 100\%$$

The average double bond contents were determined by the following equation: % modification × lysozyme residues (6 for lysozyme).

2.5. Preparation of UWPU-L coatings

UWPU-L coatings were prepared by casting 1 mL of WPUA, photoinitiator Darocur 1173 (2% w/w, based on the ionomer) and double bond modified lysozyme onto a poly (tetrafluoroethylene) (PTFE) dish with a diameter of 36 mm and drying at room temperature for 24 h. After volatilization of water the mixture was cured under a UV light provided by a lamp for 60 s with distance between the thin film samples and the centre of UV lamp of 20 cm. The lamp has main wave length of 365 nm, the power 1000 W, the UV energy per second of 1000 J/s. The reaction mechanism for the preparation of UV-curable waterborne polyurethane coating containing double bond modified lysozyme was schematically presented in Scheme 1. To determine the amount of lysozyme released from these films, the films were washed with PBS buffer and the amount of lysozyme in the washing solution was measured using the micro bicinchoninic acid (Micro BCA) assay (Pierce Biotechnology, Rockford, IL).

2.6. Coating characterization

Chemical structures of the UWPU-L coatings were characterized with an ATR-FTIR spectrometer (NICOLET iS50, Thermo Fisher Scientific, USA). The spectrogram in the 4000–400 cm^{-1} ranges was obtained with a resolution of 2 cm^{-1}. Thermogravimetric analysis (TGA) of the UWPU-L coatings was performed on SDT Q600 (TA instrument, USA). Samples of 15–20 mg were heated from room temperature to 600 °C at a heating rate of 10 °C min^{-1} under a nitrogen atmosphere. The mechanical strength of the coating films was measured by DMA Q800 (TA instrument, USA) equipped with a tensile clamp. The samples (4 mm × 4 mm × 0.1 mm) were elongated at a speed of 0.2 N min^{-1}. The contact angles of the coatings were measured by DSA100 (KRUSS, Germany).

2.7. Catalytic activity of the UWPU-L coatings

The measurement of lysozyme activity is according to previous study with some modification [29]. 15 mg of ML cells were suspended in 50 mL of PBS buffer (0.1 M, pH 6.2). 2.0 mL of the suspension was transferred to the PTFE dish (10.2 cm^{-2}) coated with UWPU-L and incubated at 40 °C. The absorbance at wavelength 450 nm was measured every 2 min for 10 min. One unit of the activity of lysozyme was defined as a decrease by 0.001 in the absorbance at 450 nm per minute. Reusability and storage stability were examined by measuring residual enzyme activities (through the above procedure) of concerned samples as a function of conditioning and reuse history.

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