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Synthesis of water-based cationic polyurethane for antibacterial and gene delivery applications

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ABSTRACT

Cationic polymers are often used as antimicrobial materials and transfection reagents. Water-based process could reduce environmental pollution and prevent the risk of solvent residue in the final product. In this study, waterborne biodegradable cationic polyurethane (WCPU) was synthesized by reacting polycaprolactone (PCL diol), isophorone diisocyanate (IPDI), and *N*-methyldiethanolamine (*N*-MDEA) under 75°C. An aqueous dispersion of WCPU nanoparticles (NPs) could be acquired by vigorous stirring under acidic condition. The particles in the dispersion had an average size of ~80 nm and a zeta potential of ~60 mV. When cast into films, the contact angle of the film was ~67° and the zeta potential was ~16 mV. WCPU NPs demonstrated excellent antibacterial activity against *Escherichia coli* (E. coli) and *Staphylococcus aureus* (S. aureus) (100% inhibition with a contact time of 3 h). Meanwhile, the antibacterial ratio of WCPU films to *E. coli* and *S. aureus* reached 100% after 24 h of contact. Moreover, WCPU NPs could be used as a transfection reagent without significant toxicity for concentrations less than 1000 μg/mL and showed the ability to condensate plasmid DNA. The transfection efficiency for HEK293T cells and hBMSCs was ~60% and ~30% at 48 h, respectively, after the transfection. Therefore, the WCPU synthesized in this study has potential antibacterial and gene delivery applications.

1. Introduction

Polyurethane has drawn a lot of research efforts because of a wide diversity of applications including in the biomedical field [1,2]. Through introducing carboxylic acid or tertiary amine into the structure, polyurethane can be synthesized and dispersed in water as ionomers with no or little solvent used [3,4]. The waterborne process reduced the environmental concerns regarding hazardous air pollutants (HAPs) and volatile organic compounds (VOCs) and the risk of toxicity arising from residual solvent.

Antibacterial activity is a notable property of medical or consumer products. Polyurethanes with antibacterial activity have been crafted into various products, e.g. textiles, wound dressings, filters [5–7]. Metal nanoparticles (NPs), such as gold and silver [6,8–10] can be blended with polyurethane to form composites that carry out antibacterial property. Cationic polymers are often antibacterial. Chitosan is one of the natural cationic polymers that has excellent antibacterial activity [11,12]. For synthetic polymers, a few cationic polyurethanes have been used in antibacterial applications [4,13]. Several other polymers were also synthesized and demonstrated antibacterial properties [14,15]. It was reported that the electrostatic interaction between cationic polymers and bacteria could lead to the disruption of bacterial membranes [16–18].

Gene therapy is a potential medical treatment for genetic diseases that transfers a specific gene sequence into the patient's cells. A viral or non-viral vector is used to carry the gene into cell nuclei. Cationic polymers are often used as non-viral carriers [19,20]. They form complexes with DNA for gene transfection. Common examples are polyesters [21,22], polyurethanes [23,24], poly(ethylenimine) (PEI) [19,25], poly(2-dimethylaminoethyl methacrylate) (PDMAEMA) [26], and poly-L-lysine (PLL) [27–29]. Polymers with biodegradable blocks have been developed as biodegradable transfection reagents [24,25,28], which can lower the risk resulted from polymer accumulation. Moreover, some biodegradable transfection reagents are designed as dissolvable in water and can be excreted through kidney in short time in order to reduce the risk from accumulation after repeating treatments [30,31]. The positive charged polymers, however, still suffer from cytotoxicity in general. A molecular weight of 25 kDa

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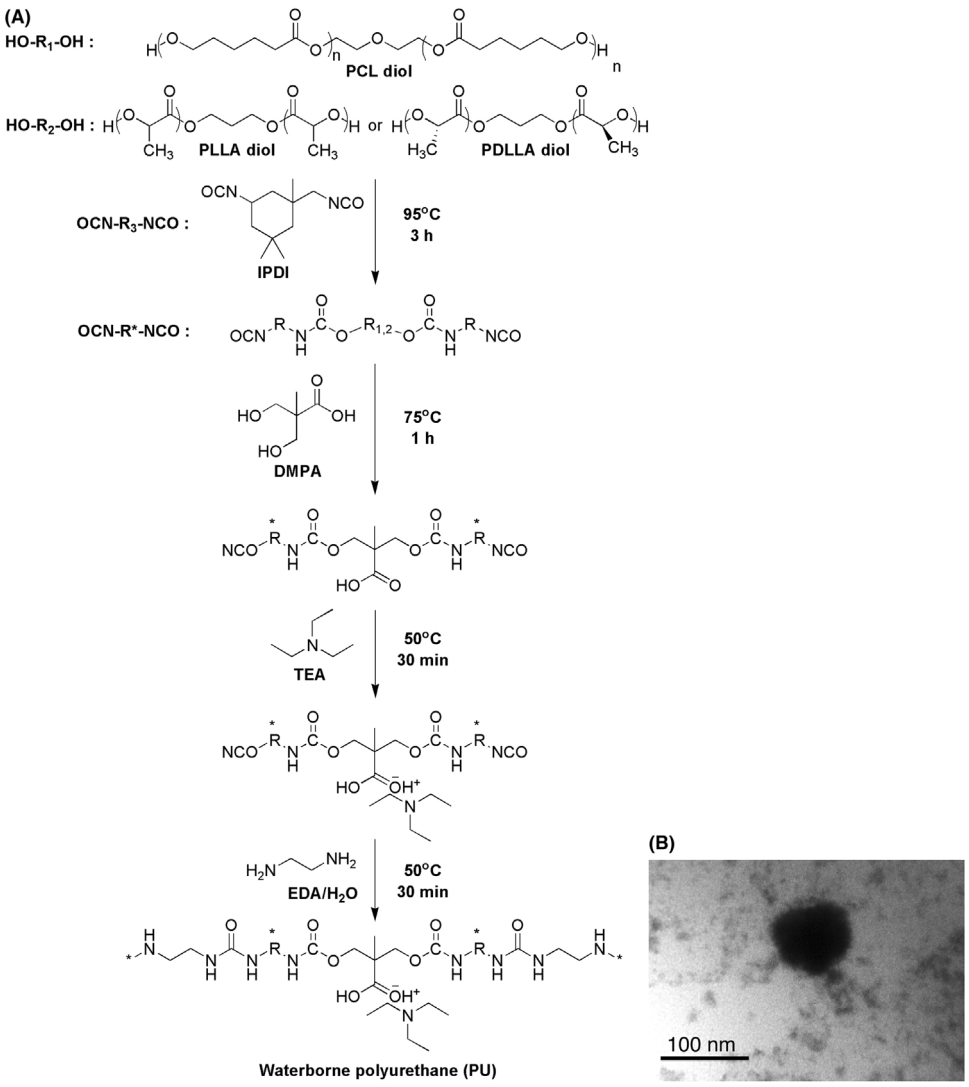

Fig. 1. (A) The synthesis scheme of water-based cationic PU (WCPU), and the structure of water-based anionic PU (WAPU) for comparison. (B) TEM images of WCPU NPs at magnification 5×10^4, and at magnification 1.5×10^5.

PEI (PEI25k) was first described by Boussif et al. [25]. This polymer shows high transfection efficiency, however, it has considerable toxicity. PEI with low molecular weight has less toxicity, but lower transfection efficiency.

Biodegradable polyurethane has been explored as a transfection reagent. Yang et al. [32] has used *N*-methyldiethanolamine (*N*-MDEA) for chain extension. Tertiary ammonium groups were incorporated both in the backbone and as a pendant group. Jian et al. [33] has copolymerized polyurethane with polyester and obtained a cationic copolymer with tertiary ammonium groups in the backbone and side chain. Chang et al. [34] grafted short branch PEI on poly(urethane-co-ester). Hung et al. [35] prepared polyurethanes containing primary amine, secondary amine, and tertiary amine in the side chain. Generally, the polyurethanes described above had lower toxicity compared to PDMAEMA or PEI. The gene delivery efficiency was comparable to the more traditional transfection reagent. These polyurethane transfection reagents, however, are difficult to synthesize and prepare as carriers, and the processes often require the use of toxic organic solvent.

Here we synthesized biodegradable cationic polyurethane nanoparticles (NPs) with tertiary ammonium group in the backbone through an eco-friendly waterborne process. The NPs and the cast films were analyzed for the antibacterial property and the NPs were evaluated for the gene delivery efficiency.

2. Materials and methods

2.1. Materials

Poly(ε-caprolactone) diol (PCL diol, Mn ~2000 Da, Sigma-Aldrich), isophorone diisocyanate (IPDI, Acros Organics) and a catalyst Tin(II) 2-ethylhexanoate (T-9, Alfa Aesar) were used for polyurethane synthesis. *N*-methyldiethanolamine (*N*-MDEA, Acros Organics) was used for chain extension. Quaternization was executed by glacial acetic acid (Sigma). Methylethyl ketone (MEK, J.T. Baker) was added to reduce the viscosity during synthesis. PCL diol, IPDI and *N*-MDEA were stored at 70 °C under reduced pressure for 24 h prior to the synthesis for water removal.

2.2. Synthesis of water-based cationic polyurethane (WCPU)

PCL idol and *N*-MDEA were added to a four-neck round-bottom flask equipped with a mechanical stirrer, thermometer, nitrogen inlet, and a reflux. The reactants were fully mixed by stirring at