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Synthesis and characterization of anti-bacterial and anti-fungal citrate-based mussel-inspired bioadhesives

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ABSTRACT

Bacterial and fungal infections in the use of surgical devices and medical implants remain a major concern. Traditional bioadhesives fail to incorporate anti-microbial properties, necessitating additional anti-microbial drug injection. Herein, by the introduction of the clinically used and inexpensive anti-fungal agent, 10-undecylenic acid (UA), into our recently developed injectable citrate-based mussel-inspired bioadhesives (iCMBAs), a new family of anti-bacterial and anti-fungal iCMBAs (AbAf iCs) was developed. AbAf iCs not only showed strong wet tissue adhesion strength, but also exhibited excellent *in vitro* cyto-compatibility, fast degradation, and strong initial and considerable long-term anti-bacterial and anti-fungal ability. For the first time, the biocompatibility and anti-microbial ability of sodium metaperiodate (PI), an oxidant used as a cross-linking initiator in the AbAf iCs system, was also thoroughly investigated. Our results suggest that the PI-based bioadhesives showed better anti-microbial properties compared to the unstable silver-based bioadhesive materials. In conclusion, AbAf iCs family can serve as excellent anti-bacterial and anti-fungal bioadhesive candidates for tissue/wound closure, wound dressing, and bone regeneration, especially when bacterial or fungal infections are a major concern.

1. Introduction

The clinical applications of biomaterials with adhesive properties as wound closure or hemostatic agents, tissue sealants, and wound dressings have advanced surgeries in terms of the facilitation of surgical operations, the improvement of patient compliance, and the reduction of healthcare costs by reducing the use of sutures, medical gauze, and other auxiliary materials [1–3]. Bacterial and fungal infections are major concerns in the use of surgical devices or implants for wound closure and wound repair, which result in prolonged wound healing, wound dehiscence, abscess formation and even sepsis, especially for applications in large area burn wound repair, wound closure for patients suffering from diabetes or other immune compromising diseases, and wound care in infection prone areas such as diabetic foot ulcers [1,4–7]. Treatment of localized fungal infections, such as osteoarticular infections, can be extremely difficult, because they take on an abscess-like or granulomatous form and relatively sequestered from circulating drugs [7]. Traditional bioadhesives typically lack native anti-bacterial and anti-fungal properties, necessitating additional anti-bacterial and anti-fungal drug injection. The inconvenience of these repeated injections and the resulting increase in cost as well as the toxicity concerns continues to limit the potential of current bioadhesives. Even if anti-microbial drugs are encapsulated in bioadhesives, sustained release is difficult to achieve and the burst release of drugs often results in undesired systemic toxicity. The development of biocompatible bioadhesives

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with intrinsic anti-bacterial and anti-fungal properties for local application in tissue/wound closure, wound dressing, or bone regeneration is urgently needed.

Biodegradable citrate-based polymers, benefitting from facile synthesis reaction and modification, excellent processing possibilities, and tailored mechanical and degradation properties, were developed in our lab for cardiovascular and orthopedic applications, as well as applications in bioimaging, drug/cell delivery, and bioadhesives [8–18]. The development of injectable citrate-based mussel-inspired bioadhesives (iCMBA) fully utilized the facile reactivity of citric acid to modify our citrate-based polymer with dopamine, a derivative of L-3, 4-dihydroxyphenylalanine (L-DOPA) that contributes to the strong under-water adhesive properties of marine mussels [19,20], through a convenient one-pot polycondensation process [18]. Citric acid is a naturally-derived organic compound that abundantly exists in citrus fruits, such as lemons and limes. As an important intermediate in the Krebs cycle that occurs in the metabolism of all aerobic organisms, citric acid is nontoxic and biocompatible [8]. As a weak organic acid, citric acid is also a natural preservative and flavoring agent used in the food industry [21]. The anti-bacterial ability of citrate-based polymers developed in our lab was also recently testified, which was attributed to that the abundant free carboxyl groups derived from citrates in the polymers may lower the local pH, suppress the nicotinamide adenine dinucleotide (NADH) oxidation, and/or chelate the metal ions in the cell wall thus altering the permeability of cell wall for nutrient uptakes to cause cell damage and then death [22].

Although citrate-based polymers possess an intrinsic anti-bacterial ability, it might be insufficient to combat wound infection without additional anti-bacterial additives. Silver and silver ions, especially silver nanoparticles that have relatively large surface areas to contact with bacteria or fungi, have been widely studied and utilized as effective anti-microbial agents [6,23,25]. Polymers containing catechol groups (from dopamine) have the ability to transform silver nitrate into silver nanoparticles by oxidation–reduction reaction and can be used for anti-bacterial purposes, as proven by previous studies [25]. Additionally, water-soluble oxidants such as sodium (meta) periodate (PI), used in our iCMBA cross-linking process, have been commonly used as anti-microbial agents in aquaculture.

In the present work, 10-undecylenic acid (UA), used as an anti-fungal agent against *Panneuritis epidemica*, was conjugated to citric acid to create a new anti-fungal citric acid (U-CA, Scheme 1A). Through a convenient one-pot polycondensation reaction of citric acid, anti-fungal citric acid (U-CA), L-dopamine, and hydrophilic poly (ethylene glycol) (PEG), a water soluble anti-fungal iCMBA pre-polymers (AiCs) were obtained. The cross-linking of AiCs was conducted by the addition of silver nitrate or sodium (meta) periodate (PI), which led to dual-functional anti-bacterial and anti-fungal iCMBA bioadhesives (AbAf iCs). The cytocompatibility of these bioadhesives was studied against human-derived mesenchymal stem cells (hMSCs). The short-term and long-term anti-bacterial and anti-fungal performances of these AbAf iCs were also thoroughly investigated using two kinds of bacteria, *Staphylococcus aureus* (*S. aureus,* from ATCC) as a representative Gram-positive bacteria and *Escherichia coli* (*E. coli,* from ATCC) as a representative Gram-negative bacteria, and *Candida albicans* (*C. albicans*) as a representative fungi that is often found in diabetic foot infections.

2. Experimental section

2.1. Materials

All chemicals were purchased from Sigma–Aldrich and used without further purification, except where mentioned otherwise.

2.2. General measurements

^{1}H-NMR spectra of monomer and pre-polymers in DMSO-d_6 were recorded on a 300 MHz Bruker DPX-300 FT-NMR spectrometer. Fourier transform infrared (FTIR) spectra were measured with a Nicolet 6700 FTIR spectrometer. Sample solutions in acetone were cast onto KBr plates, with blank KBr used as background. UV–vis spectra were recorded using a UV-2450 spectrometer (Shimadzu, Japan) with a minimum wavelength resolution of 0.2 nm.

2.3. Polymer syntheses

2.3.1. Anti-fungal citric acid monomer (U-CA) synthesis

A new anti-fungal citric acid monomer was synthesized by modifying citric acid (CA) with 10-undecylenic acid (UA) via a method similarly described elsewhere (Scheme 1A) [26]. Briefly, citric acid (9.606 g, 0.05 mol) and zinc chloride ($ZnCl_2$, 0.6815 g, 0.005 mol, 0.1 eq. to CA) were added into 10-undecenoyl chloride (21.5 mL, 0.1 mol) in a dried 100-mL round-bottom flask. The reaction mixture was heated at 90 °C with stirring for 24 h. After cooling down to room temperature, diethyl ether (100 mL) was added to the mixture, and the solution was poured into ice water (50 mL) with stirring. The organic portion was separated, dried over anhydrous sodium sulfate, and the solvent was then evaporated. The crude product was purified by precipitation in hexane (250 mL). The product, 10-undecylenic acid modified citric acid (U-CA), was obtained as viscous dark brown oil (12.7 g, 71% yield). ^{1}H NMR (300 MHz; DMSO-*d6*; δ, ppm) of U-CA: 1.25–1.35 (s, $OCOCH_2CH_2-(C\mathbf{H}_2)_5-$ from UA), 1.52 (s, $OCOCH_2C\mathbf{H}_2-$ from UA), 1.98–2.05 (m, $CH_2{=}CH{-}C\mathbf{H}_2-$ from UA), 2.25–2.30 (m, $OCOC\mathbf{H}_2-$ from UA), 2.65–3.00 (m, $-C\mathbf{H}_2-$ from CA), 4.91–5.02 (m, $C\mathbf{H}_2{=}$ CH– from UA), 5.74–5.84 (m, $CH_2{=}C\mathbf{H}-$ from UA). FTIR of U-CA (cast film on KBr, cm^{-1}): 1897 ($-\underline{CH_2}-$) and 1733 ($\underline{COO}R$).

2.3.2. Anti-fungal iCMBA pre-polymers (AiCs) synthesis and characterization

Anti-fungal iCMBA (AiC) pre-polymers were synthesized by polycondensation of citric acid (CA), U-CA, poly(ethylene glycol) (PEG), and catechol-containing compounds, such as dopamine and L-DOPA (L-3,4-dihydroxyphenylalanine), as illustrated in Scheme 1B, adapting a method used in our previous work [18]. Briefly, CA (17.29 g, 0.09 mol), U-CA (7.18 g, 0.02 mol) and PEG200 (with an average molecular weight of 200Da, 20 g, 0.10 mol) were placed in a 100-mL round-bottom flask and heated to 160 °C until a molten, clear mixture was formed under stirring. Then the temperature was reduced to 140 °C, followed by the addition of dopamine (5.69 g, 0.03 mol) under N_2 atmosphere. The reaction was continued under vacuum for approximately 6 h until the stir bar twitched at 60 rpm. Then the reaction was stopped and the pre-polymer was dissolved in deionized (DI) water and purified by extensive dialysis using a dialysis tube with a molecular weight cut-off (MWCO) of 500 Da. After freeze-drying of the dialyzed solution, AiC-P_{200} pre-polymer was obtained. By adjusting the molecular weight and architectural structure (linear or branched) of PEG used, different AiC pre-polymers were synthesized as shown in Table 1. The feeding amount of dopamine was fixed at the ratio of 1.1/0.3 ((CA+U-CA)/dopamine). FTIR of AiC (Fig. 1A, by casting polymer solution in acetone on KBr, cm^{-1}): 1898 ($-\underline{CH_2}-$) and 1734 ($\underline{COO}$-), 1633 ($\underline{CO}NH-$). Representative ^{1}H NMR (Fig. 1B, 300 MHz; DMSO-d_6; δ, ppm) of AiC pre-polymer: 1.22 (s, $OCOCH_2CH_2-(C\mathbf{H}_2)_5-$ from UA), 1.47 (s, $OCOCH_2C\mathbf{H}_2-$ from UA), 1.65 (m, $CH_2{=}CH{-}C\mathbf{H}_2-$ from UA), 2.25–2.30 (m, $OCOC\mathbf{H}_2-$ from UA), 2.65, 3.05 (m, $-C\mathbf{H}_2-$ from CA and citric acid of U-CA), 4.77 (broad, $C\mathbf{H}_2{=}$CH– from UA), 5.34