Antibacterial Nanoparticles with Universal Adhesion Function Based on Dopamine and Eugenol

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Abstract: In this work, dopamine methacrylamide (DMA) and eugenyl methacrylate (EMA) were used to synthesize polymeric particles of Poly (DMA-co-EMA) by free radical precipitation copolymerization. These two monomers were modified from dopamine (consisting of the catechol moieties adhering to various materials) and eugenol (with antibacterial property), respectively. The proton nuclear magnetic resonance (1H NMR) and Fourier transform infrared (FT-IR) spectroscopy were applied to confirm the successful synthesis of the two monomers and copolymer. The scanning electron microscope (SEM) images showed the size and morphology of the polymer particles. The results indicated that regular particles with uniform size could be obtained with a monomer feeding ratio of 5:5. The results of antibacterial activity test indicated that the obtained polymer particles have an antibacterial rate over 90% to *Eugenia coli*.

Keywords: antibacterial; universal adhesion; particle, precipitation polymerization

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1 Introduction

Nowadays, implant materials are widely used in biomedical applications, such as bone tissue substitute, vascular prosthesis, and artificial heart valve. However, the long-term or short-term indwelling of biomaterials in human body often leads to bacterial infections, which are critical issues and may lead to 20% of implant failures (Hendriks et al., 2004). Bacterial infections related to implants are complex processes and mainly consist of initial bacterial adhesion, biofilm formation and infections (Costerton, 1999). Bacterial infections are difficult to treat, therefore it is necessary to endow an antibacterial surface onto implant materials.

Successful fabrication of an antibacterial surface can be achieved by inhibiting the adhesion of bacterial through some antifouling materials like polyethylene glycol (PEG) (Ding et al., 2012; Shalumon et al., 2018) and zwitterionic polymers (Lowe et al., 2002; Biehl et al., 2018), which can form a hydration layer as an effective barrier. However, such strategy can only avoid the adhesion and is useless for those have already adhered to the surface. The introduction of antibacterial agents such as chitosan (Rinaudo, 2007), quaternary ammonium salt (Li et al., 2013) and silver (Dai et al., 2018) can solve this problem through killing them in contact. From all the antibacterial agents reported, eugenol is an effective natural antibacterial agent extracted from *Eugenia caryophyllata*. Eugenol has been widely used for its antibacterial activity and biocompatibility, which has been confirmed in previous studies (Laekeman et al., 1990; Yang et al., 2015).

Compared with antibacterial agents with small molecules, antibacterial polymers are more stable and less toxic, and the morphology in nanoparticles brings more advantages than the linear ones (Lam et al., 2018), thus antibacterial polymeric nanoparticles (APNs) gain more and more interests. The superiority of the APNs is due to their unique size effect and highly specific surface area (the total area of a material per unit mass and an importance parameter of nanoparticle), which both make stronger interaction between materials and bacteria (Lam et al., 2018). Two ways are often used to form these APNs, one is composed of an inorganic core with a polymer shell (Wang et al., 2012; Le Ouay et al., 2015; Yuan et al., 2016), and the other is formed through the self-assembly of polymers with inherent antibacterial ability (Coady et al., 2014; Nguyen et al., 2017). However, only few studies introduce the APNs into an antibacterial surface and how to realize stable immobilization should be taken into consideration. For this point, it is well known that dopamine is an appropriate candidate to act as adhesive ligand (Lee et al., 2007; Zhao et al., 2017).

In this study, eugenol and dopamine were used to fabricate nanoparticles which had satisfied antibacterial and adhesive properties to be utilized in coating materials for implants. After chemical modification of dopamine and eugenol, they are functionalized with vinyl, which are...
easily to be polymerized with the method of precipitation polymerization. Precipitation polymerization is applied due to its simple process without any stabilizer compared to other common methods (Letícia Braz et al., 2017). Further, the adhesion and antibacterial properties of fabricated nanoparticles were investigated.

2 Materials and Methods

2.1 Materials

Dopamine hydrochloride (DA-HCl, 99%), methacrylic anhydride (MAA, 94%) were both purchased from Sigma-Aldrich. Eugenol (98%), trimethylamine (99.5%), and methacryloyl chloride (95%) were purchased from J&K Chemical. Sodium tetraborate (99%), sodium carbonate (99%), sodium hydroxide (NaOH, 96%) and azodiisobutyronitrile (AIBN, 99%) were all purchased from Aladdin. Ethyl acetate (99.5%), tetrahydrofuran (THF, AR), hexane (99%), diethyl ether (AR), hydrochloric acid (HCl, AR), ethyl alcohol (AR), acetone (AR) were purchased from Xilong Scientific. The AIBN was purified by recrystallization in ethyl alcohol twice before use. The NaOH was dissolved in deionized water to form NaOH solution, and HCl was diluted to 6 mol/L by deionized water.

2.2 Synthesis of dopamine methacrylamide (DMA)

The DMA was synthesized from DA-HCl according to a previously described procedure with slight modifications (Glass et al., 2009). Briefly, sodium tetraborate (10 g) and sodium carbonate (4 g) were dissolved in 100 mL of deionized water to protect dihydroxy benzene moiety of the DA with bubbling with N₂ for 20 min to remove O₂. After that, DA-HCl (5 g) was added and stirred until form a homogeneous solution, followed by dropwise addition of the MAA (4.7 mL) pre-dissolved in 5 mL of the THF. The pH of solution was kept above 8 with the addition of 1 mol/L NaOH throughout the reaction. After 14 h of reaction with continuous stirring and N₂ bubbling in room temperature, a white suspension was obtained. Then, after vacuum filtered and acidizing to pH=2 with 6 mol/L HCl, the obtained aqueous solution was washed three times with 50 mL of ethyl acetate. The organic layers were combined and dried over MgSO₄. After reduced to 25 mL with a rotary evaporator, the solution was added to 250 mL of hexane with vigorous stirring and then the formed suspension was refrigerated in 4°C overnight to maximize crystal formation size. Finally, after drying and purification by recrystallization from hexane, the DMA was successfully prepared. The chemical structure of the DMA was characterized by ¹H NMR and FT-IR spectroscopy. The ¹H NMR spectrum (δ: ×10⁻⁶, 400 MHz, DMSO-d₆): 6.48–6.65 (Ph, m, 3H); 5.61 (CH₂=CH—, d, 1H); 5.29 (CH₂=CH—, d, 1H); 3.52 (CH₂—Ph, t, 2H); 1.83 (CH₂—Ph, t, 2H); 1.38 (CH₂—, s, 3H).

2.3 Synthesis of eugenyl methacrylate (EMA)

Eugenol (6.56 g) and trimethylamine (4.04 g) were dissolved in 100 mL of diethyl ether and bubbled with N₂ for 20 min. Then, methacryloyl chloride (4.7 g) dissolved in 25 mL of diethyl ether was added dropwise with constant stirring. The mixture solution was stirred at room temperature and under nitrogen atmosphere for 48 h. The obtained yellow suspension was filtered and washed with 5% NaOH and deionized water for three times respectively. After drying over MgSO₄, the solvent was removed by rotary evaporator, and then the EMA was successfully prepared. The chemical structure of the EMA was characterized by ¹H NMR and FT-IR spectroscopy. The ¹H NMR spectrum of the product was in accordance with previous literature (Rojo et al., 2006) (δ: ×10⁻⁶, 400 MHz, CDCl₃): 6.98–6.76 (Ph, m, 3H); 6.35, 5.73 (CH₂=CH—, 2s, 2H); 5.97, 5.09 (CH=CH₂, m, 3H); 3.79 (CH₃OPh, s, 3H); 3.38 (CH₂Ph, s, 2H); 2.06 (CH₃—, s, 3H).

2.4 Synthesis of DMA-co-EMA copolymer Poly (DMA-co-EMA)

As shown in Fig. 1, Poly (DMA-co-EMA) copolymers were synthesized by free radical precipitation polymerization of the DMA and EMA with the AIBN as an initiator.
Briefly, the DMA and EMA with different ratio (7:3, 5:5, and 3:7) were degassed with vacuum pump for 20 min in reaction tube respectively. And the AIBN with solvent mixture consisting of ethyl alcohol/deionized water (V:V=1:9) pre-bubbled with N₂ for 20 min was added under nitrogen atmosphere. Then, after reacted at 70 °C for 8 h in dark, crude product was obtained by being precipitated in deionized water and centrifuged. The final products were white powders after freeze drying for 24 h and named as PD₇E₃, PD₅E₅ and PD₃E₇ respectively. The chemical structure of polymer particles was characterized by FT-IR spectroscopy. Size and morphology were characterized by SEM.

2.5 Adhesion and antibacterial activity test
A series of substrates were taken to characterize the universal adhesion function of Poly (DMA-co-EMA) particles. All substrates were ultrasonically cleaned with deionized water, ethyl alcohol and acetone in sequence, and dried with N₂. After being immersed in polymer solution (1 mg/mL, Tris-buffered saline, pH 8.5) for 24 h, substrates were rinsed and dried. More details were observed by the SEM.

The antibacterial activity of Poly (DMA-co-EMA) particles was assessed toward E.coli using the minimal inhibitory concentration (MIC) method. Briefly, particles were dispersed in 1 mL of bacterial inoculum (10⁶ CFU per mL) and diluted using double broth dilution method, the final concentration was 40, 20, 10, 5, 2.5 mg/mL, respectively. All samples were incubated for 24 h at 37°C with shaking at 150 r/min. The number of survived bacteria after co-cultured with Poly (DMA-co-EMA) was determined by plating 100 μL of the suspensions onto agar plat and further incubation for 24 h at 37°C. Bacterial inoculum without Poly (DMA-co-EMA) served as control. Plate counting method was used to calculate the antibacterial rate with a formula as follows (Cockerill, 2013):

\[
R = \frac{N_0 - N_t}{N_0} \times 100\%
\]

where \(N_0\) represents the plate counting results of the control group, \(N_t\) represents the plate counting results of the experimental group, \(R\) is the antibacterial rate, and MIC is the lowest concentration when \(R\) reaches 90%.

3 Results and Analyses

3.1 Characterizations of Poly (DMA-co-EMA) particles
The Poly (DMA-co-EMA) copolymer was prepared by free radical precipitation polymerization, and all the reaction conditions were determined by our preliminary experiments. A series of Poly (DMA-co-EMA) copolymers were obtained by adjusting the molar ratio of the DMA to EMA. The successful synthesis of Poly (DMA-co-EMA) is confirmed by the FT-IR spectroscopy, and the FT-IR spectrum of the Poly (DMA-co-EMA), DMA, EMA, DA-HCl, and eugenol are all shown in Fig. 2 for a clear comparison.

Fig. 2 The FT-IR spectra of dopamine and DMA (a); eugenol and EMA (b); DMA, EMA and Poly (DMA-co-EMA) (c)

As shown in Fig. 2a, the characteristic signal at 1653 cm⁻¹ is attributed to the stretching vibration of C=O in amide group, which can only be observed in the spectrum of the DMA, indicating that amidation occurred. In addition, peaks around 3225 cm⁻¹ exhibit the stretching vibration of O–H in catechol group, which indicates that the adhesion function can be retained. In Fig. 2b, the exist of C=O in ester bond at the peak of 1737 cm⁻¹ and disappearance of O–H in phenolic hydroxyl group at peaks of 3550 cm⁻¹ show esterification between eugenol and methacryloyl chloride. The characteristic signals of
both monomers can be found in the spectrum of Poly (DMA-co-EMA) (Fig. 2c) at 3416 cm⁻¹ and 1749 cm⁻¹, indicating that the DMA was copolymerized with EMA successfully.

The size and morphology of the Poly (DMA-co-EMA) particles were characterized by the SEM and results are shown in Fig. 3. The SEM images show that monomer ratio exerts little influence on the size of the Poly (DMA-co-EMA) particles. The size has a slightly decrease from 339 nm to 310 nm when the ratio changes from 7:3 to 5:5, and when ratio further decreases to 3:7, the aggregation of particles makes it hard to calculate the size. It is related to the formation process of the copolymer particles, and the growth of these particles may be similar to the typical process as follows (Downey et al., 1999): monomers and initiator were completely dissolved in solvent prior to the start of the reaction, and after initiated by heating, oligomers were formed and precipitated because of reduction in solubility, then the oligomers were gathered and intertwined to form stable nucleus, these nucleus captured monomers and oligomers from the solution to further polymerize until form the final particles. The decrease in size as ratio decrease may be attributed to the hydrophobicity of the EMA, which makes it easier for the oligomers to precipitate from the solution to form stable nucleus in short time, and the increase of stable nucleus will inevitably lead to a decrease in the size of the final particles. In addition, the morphology of polymer particles is also affected by monomer ratio. The morphology becomes more regular and uniform as ratio changes from 7:3 to 5:5, but nonspherical particles can be found when ratio reaches to 3:7 because of the aggregation of particles as hydrophobicity increases. Meanwhile, when the ratio further goes down to 0:10, conspicuous bulk instead of particles is observed. Therefore, polymer particles with regular morphology and appropriate size can be obtained at the monomer ratio of 5:5.

![Fig. 3](image)

**Fig. 3** The SEM images of Poly (DMA-co-EMA) particles with different monomer ratios of 7:3 (a), 5:5 (b) and 3:7 (c)

### 3.2 Adhesion and antibacterial activity test of Poly (DMA-co-EMA) particles

To test the adhesion property of polymer particles with different monomer ratios, titanium (Ti) plates were used as the substrate. After immersed in polymer solution, every plate was rinsed by phosphate buffered saline (PBS) to remove any uncoated particles. The adhesion condition was revealed by the SEM and results are shown in Fig. 4. Compared with pure Ti plate, all the three types of particles coated on the surface successfully with some difference. With more catechol group, PD-E₃ particles stack together to form a very thick and tight coating on the surface (Fig. 4b). As the content of the DMA decreases, a monolayer formed (Fig. 4c). And with the least content of the DMA, there are only few particles which can be found on the surface (Fig. 4d). It is obvious that the adhesion capacity of the Poly (DMA-co-EMA) particles is strongly related to the content of the DMA, thus particles with a monomer ratio of 7:3 show the best adhesion ability.

The Ti plates, silicon wafers and polydimethylsiloxane (PDMS) were used to certify the universal adhesion function of the Poly (DMA-co-EMA) particles. Results shown in Fig. 5 demonstrate that the Poly (DMA-co-EMA) particles adhere to all the three substrates successfully, indicating the modification of dopamine retains the universal adhesion function.

![Fig. 4](image)

**Fig. 4** The SEM images of pure Ti plate (a) and Ti plate with adhesion of Poly (DMA-co-EMA) at monomer ratio of 7:3 (b), 5:5 (c) and 3:7 (d)
The Gram-negative E. coli was used to evaluate the antibacterial activity of the Poly (DMA-co-EMA) particles by using the MIC and plate counting methods. As shown in Fig. 6, the PD3E3 particles show an unsatisfied anti-E. coli performance with an antibacterial rate lower than 60%. By contrast, PD5E5 and PD3E7 particles are more efficient in dealing with E. coli with high antibacterial rate over 90%. It can be easily explained by the increase of the EMA, which works as antibacterial agent. In addition, it’s unexpected to find that there is just slight difference between the PD3E5 and the PD3E7, which does not match the growth of the EMA.

We infer that the aggregation of particles hides the antibacterial groups and thus makes it hard to form contact between antibacterial groups and bacteria, which is important for the Poly (DMA-co-EMA) particles to kill them. Overall, the antibacterial rate calculated by the formula (in section 2.5) indicates that the MIC of the PD3E3 and the PD3E7 particles is around 20 mg/mL. Compared with eugenol, the decrease of antibacterial effect shows the obvious influence of modification to eugenol, and the reason needs to be further discussed.

4 Conclusions
Polymeric nanoparticles of the Poly (DMA-co-EMA) with antibacterial and universal adhesion function were successfully synthesized from vinyl modified dopamine and eugenol through easily precipitation polymerization. The results of 1H NMR and FT-IR spectroscopy analysis confirmed the successful synthesis of the Poly (DMA-co-EMA). And the results of the SEM images indicated that polymer particles with regular morphology and uniform size can be found when the ratio of the DMA to EMA is 5:5. From a comprehensive perspective, the Poly (DMA-co-EMA) particles with a monomer ratio at 5:5 presented excellent properties in adhering to a variety of materials and possessed a high antibacterial rate over 90% to E. coli, and they may have potential applications in antibacterial coating on implant materials to prevent bacterial infections.

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