Polymer 83 (2016) 223–229

Contents lists available at ScienceDirect

Polymer

journal homepage: www.elsevier.com/locate/polymer

Antibacterial performance of various amine functional polymers coated silica nanoparticles

Inkyu Lee, Jongmin Roh, Jungsup Lee, Jooyoung Song, Jyongsik Jang*

School of Chemical and Biological Engineering, Seoul National University, 599 Gwanangro, Gwanak-gu, Seoul 151-742, South Korea

ARTICLE INFO

Article history:
Received 10 October 2015
Received in revised form
11 December 2015
Accepted 18 December 2015
Available online 20 December 2015

Keywords:
Amine
Antimicrobial
Core/shell

ABSTRACT

Five types of amine functional polymer shells were formed on the surface of silica nanoparticles *via* vapor deposition polymerization and oxidation polymerization. Scanning electron microscopy, transmission electron microscopy, elemental analysis, and Fourier transform infrared spectroscopy have been used to characterize the resulting various types of amine functional polymers coated silica nanoparticles. Electron microscopy studies reveal that the thin polymer shell is successfully formed on the silica surface. The antibacterial performance of the prepared core/shell nanoparticles was investigated against both Gram-positive (*Escherichia coli*) and Gram-negative (*Staphylococcus aureus*) bacteria. The various amine functional polymers coated silica nanoparticles presented antibacterial activity against both bacteria. In contrast, silica/PPy core/shell nanoparticles had no bactericidal efficiency, because the amine group of PPy does not provide protonated nitrogen atoms which can kill the bacteria. The obtained results evidence that antimicrobial activity of amine functional polymers is influenced by the state of the amine groups than positively charged amino groups.

1. Introduction

With increasing concerns of infections resulting from harmful bacteria, there are growing demands for the preparation of effective and sustainable antimicrobial agents [1–6]. Most commercial antibacterial materials exhibit bactericidal properties *via* releasing of the impregnated biocides such as silver or silver ions, chlorine, and diverse antibiotics [7–10]. Although these materials present excellent bactericidal activities, the released biocides could induce the development of bacterial resistance, which is one of the serious problems modern medicine faces. Additionally, the continuous leaching of biocides will eventually lead to depletion of the antibacterial performance [11,12].

Contact-active biocidal materials, which could kill the bacteria through direct-contact with the bacterial membrane rather than releasing biocides, have attracted a great deal of interest due to their eco-friendly activities and long-term durability [13]. Quaternary ammonium compounds, rhodanine derivatives, and phosphonium salts are categorized as contact-type biocides [14–19]. Among them, the quaternary ammonium compounds have been widely studied in antibacterial fields because these compounds have low toxicities to human being and excellent antibacterial performances [20]. Chan-Park and co-workers synthesized the highly antimicrobial surfaces based on contact-active hydrogel layer made of quaternized ammonium chitosan. The hydrogel coated surfaces presented antimicrobial and biocompatible properties [21]. Recently, Dong et al. demonstrated the synthesis of quaternized amino group having polymer (quaternized poly(2-(dimethylamino)ethyl methacrylate)) coated magnetic nanoparticles by ATRP method [22]. These nanoparticles showed excellent recyclable antibacterial properties against *Escherichia coli*. It is well-known that the quaternary ammonium compounds can interact with negatively charged bacteria membrane and cause bacterial cell death *via* disturbing the bacterial metabolism.

On the other hand, some polymers which have non-quaternized amino groups also show antibacterial performance because these amino groups are protonated to obtain positive charge which could kill the bacteria. Gellman and co-workers synthesis the tertiary amine functioned polystyrene and it showed excellent bacteria killing properties against both Gram-positive and Gram-negative bacteria [23]. In addition, our group previously reported that the poly(2-tert-butylaminoethyl) methacrylate (which has antibacterial amino group in its side chain) can effectively kill the bacteria when the size of the polymer particles are decreased in nano-meter

* Corresponding author.
E-mail address: jsjang@plaza.snu.ac.kr (J. Jang).

http://dx.doi.org/10.1016/j.polymer.2015.12.032
0032-3861/

scale [24]. There have been numerous efforts to prepare the nano-sized antibacterial agents using positively charged amine compounds owing to their high bactericidal efficacy based on the enlarged surface area and relatively biocompatible properties. Therefore, there is a necessity for a better understanding of the factors affecting the antibacterial activities of various polymers nanoparticles which possess positively charged amino group.

Herein, the various types of amine functional polymers were introduced to the surface of silica nanoparticles *via* surface-initiated polymerization method in order to comparatively investigate their antimicrobial properties. To test their antibacterial properties of the synthesized core/shell nanoparticles, *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) were selected as Gram-negative and Gram-positive bacteria, respectively. In this study, dependency of the antibacterial properties on the state of amine groups was investigated. Additionally, the contact-attributable bactericidal performances of the prepared nanoparticles were studied using a bactericidal test.

2. Materials and methods

2.1. Materials

The monomers; pyrrole, diallylamine, 2-(tert-butylamino)ethylmethacrylate (TBAM), *N*,*N*-dimethylaminoethyl methacrylate (DMAEMA), and ethylene glycol dimethacrylate (EGDMA) were obtained from Aldrich (St. Louis, MO). All monomers and EGDMA were used after purification using inhibitor remover. Tetraethyl orthosilicate (TEOS), ethanol, ammonia solution 28.0–30.0%, dimethylformamide (DMF), 1-bromohaxane, $FeCl_3$, glutaraldehyde, osmium tetroxide and chlorodimethylvinylsilane (CDVS) were also obtained from Aldrich (St. Louis, MO). The initiator 2,2'-azobis-(isobutyronitrile) (AIBN) was purchased from Junsei Chemical Co.,Ltd. (Japan).

2.2. Fabrication of the 50 nm silica nanoparticles

The 50 nm silica nanoparticles were prepared by using Stöber method. Tetraethyl orthosilicate (3 mL) was added to a mixture of deionized water (12 mL), ethanol (74 mL) and ammonia (3 mL). The mixture was stirred for 12 h at 35 °C. Then 50 nm silica nanoparticles were washed with excess deionized water 3 times.

2.3. Fabrication of silica/polypyrrole core/shell nanoparticles

To synthesize silica/polypyrrole(PPy) core/shell nanoparticles, Fe (III) ions on the silica nanoparticles are necessary as oxidant. 1 g of 50 nm silica nanoparticles were dispersed in 100 mL of $FeCl_3$ aqueous solution (6.2 mmol) and vigorously stirred at room temperature for 24 h. After centrifugation, Fe (III)-ion-treated silica nanoparticles were obtained and dried in a vacuum oven at 25 °C. Then, 0.5 g of Fe (III)-ion-coated silica nanoparticles were dispersed in 100 mL of hexane using sonication for 20 min. The pyrrole monomer (0.75 mmol) was injected to the reaction medium and the chemical oxidation polymerization of pyrrole proceeded for 12 h on the surface of silica nanoparticles at 25 °C with vigorous stirring. After polymerization, silica/PPy core/shell nanoparticles were obtained by centrifugal precipitation and washed with excess deionized water to remove residual reagents.

2.4. Surface-modifying of the 50 nm silica nanoparticles

The silica colloids of 50 nm diameter were pretreated with chlorodimethylvinylsilane (CDVS) in a water–ethanol mixture solution (4: 1 v/v) for 24 h to improve the chemical affinity of silica to organic monomers. After washing with excess deionized water 3 times to remove the residual reagents, the surface-modified silica nanoparticles were dried in a vacuum oven at 25 °C.

2.5. Fabrication of silica/secondary and tertiary amine functional polymer core/shell nanoparticles by using vapor deposition polymerization (VDP) method

0.1 g of 50 nm silica nanopowder treated with CDVS and 0.01 g of 2,2'-azobisisobutyronitrile (AIBN) as a radical initiator were blended into the reactor, then the reactor was evacuated to *ca.* 10^{-1} torr at 25 °C. Under these vacuum conditions, 0.5 mL of mixed each monomer (diallylamine/TBAM/DMAEMA) and EGDMA (4: 1 v/v) was injected into the reactor and utterly vaporized at 50/80/50 °C respectively. By a hydrophobic interaction, the monomer vapor was physically adsorbed on the surface of the CDVS-treated silica nanoparticles. After the polymerization process for 24 h, the amine functional polymer-coated silica nanoparticles were obtained by venting the reactor to discard excess monomer vapor. The final product of core/shell nanoparticles was washed with ethanol to remove the residual reagents.

2.6. Fabrication of silica/quaternary amine functional polymer core/shell nanoparticles by quaternization reaction

The silica/poly(DMAEMA-*co*-EGDMA) core/shell nanoparticles was fabricated according to the typical procedure. The 0.3 mg of silica/poly(DMAEMA-*co*-EGDMA) core/shell nanoparticles was added to 4 mL of DMF and dissolved by stirring at 70 °C. 4 mL of DMF solution of 1-boromohexane (2.4 mM) was added to prepared solution *via* drop-wise method. Then the quaternization reaction was conducted at 70 °C for 48 h. After reaction, the mixture was lowered to room temperature and collected by centrifugation. The obtained silica/quaternized polyDMAEMA core/shell nanoparticles were washed with acetone and deionized water 3 times.

2.7. Antibacterial tests

The *E. coli* and *S. aureus* suspensions were prepared for the bacteria tests (containing 10^6–10^7 colony forming units (CFU) per each milliliter). The pH of the test medium was *ca.* 7.0. The as-prepared silica/various amine functional polymer core/shell nanoparticles dispersed in distilled water (10 mg in 1 mL) were injected with 50 μL of the bacterial suspension (*E. coli* and *S. aureus*) for the antibacterial test. The distilled water (without samples) and pristine silica nanoparticles were prepared for the control experiment and comparative data, respectively. The shaking incubator was maintained at 37 °C with 200 rpm to incubate the five different tube of bacteria-inoculated solutions. After a specific time, 100 μL aliquots were taken from each tube and cultured on LB agar plates. Then, these LB agar plates were kept at 37 °C for 24 h in the incubator. The all-grown bacterial colonies were counted to evaluate antibacterial efficiency. It was repeated three times and the results were averaged for the accuracy of the bacterial test data. Field-emission scanning electron microscopy (FE-SEM) was used to observe the bacterial morphologies. The bacterial suspension with nanoparticles was drop-casted on the surface of the silicon wafer and cultivated for 2 h at 37 °C. Then, the bacteria on the silicon wafer were fixed in 2.5% glutaraldehyde for 2 h and rinsed with distilled water. Post-fixation proceeded with 1% osmium tetroxide in distilled water for 1 h. After these treatments, the samples were dehydrated with series ethanol solution (20–100%) and coated with platinum/palladium for FE-SEM observation.