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Application of silver nanodots for potential use in antimicrobial packaging applications

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ABSTRACT

A simple method to fabricate well-defined silver nanodots of different sizes using self-assembled polystyrene-b-poly(ethylene oxide) (PS-b-PEO) block copolymer was developed. The most well-defined nanodot patterns were observed using optimal concentrations of silver precursor (0.4, 0.6, and 1.2%) with average sizes of 10, 18, and 28 nm by different molecular weights of PS-b-PEO. Silver nanodot patterns were not observed at higher Ag precursor concentrations. In addition, after repeated depositions, the antimicrobial activity (AA) towards bacteria increased compared to well-defined nanodot arrangements. The AA of the silver nanodots was significantly affected by the concentration used independent of the particle size of the silver nanodots. Potentially, silver nanodots can be used as antimicrobial packaging application to preserve the quality of food products due to the data generated here demonstrated that these materials significantly delayed the growth of *Pseudomonas fluorescens* and *Staphylococcus aureus*.

Industrial relevance: Food wastage is a significant cost to industry and society as a whole and impacts all stages of the food distribution cycle from transport and storage to shelf life and end-consumer use. Antimicrobial packaging could significantly decrease product decomposition and add value for producers by preserving product shelf life. Metal-based nanoparticles (NPs) (especially Ag) have previously been identified as potential antimicrobials but their performance is dependent on factors such as size and shape, concentration, morphology, composition and crystallinity. Their use in packaging has been limited partly by issues such as size control, powder handling, surface attachment and application to polymer films which can be challenging. We developed a novel method for generating antimicrobial surfaces based around the self-assembly of a polystyrene-b-polyethylene (PS-b-PEO) block copolymer that is a simple, effective and efficient method for generating highly uniform size and shape defined NPs (as nanodots) on a surface in a well-defined arrangement without the need of expensive lithographic techniques. The developed silver nanodot surfaces exhibited good antimicrobial activity against Gram-positive and Gram-negative bacteria and potentially can be used in antimicrobial packaging applications.

1. Introduction

Silver (Ag) nanoparticles (NPs) and Ag ions (Ag^+) have received considerable attention in applications as diverse as micro-electronics, catalysis and reaction intermediates due to their unique chemical and physical properties (Lei, Calata, Lu, Chen, & Luo, 2010). They are also widely used in textiles, water filtration, health care and food packaging because of their outstanding antimicrobial properties (Boisselier & Astruc, 2009; Edwards-Jones, 2009; Huang, Wang, Wu, & Lü, 2010; Larimer, Ostrowski, Speakman, & Nettleship, 2010; Lei & Ju, 2012; Martínez-Abad, Lagaron, & Ocio, 2012; Osório, Igreja, Franco, & Cortez, 2012). Metal-based NPs (especially Ag) have been identified as potential antimicrobials, but their performance is dependent on factors, such as size and shape, concentration, morphology, composition and crystallinity (Cruz-Romero, Murphy, Morris, Cummins, & Kerry, 2013). However, the synthesis and control of the particle size of the NPs, other than the attachment to a polymer surface can be challenging. This paper reports a novel methodology of generating antimicrobial surfaces using nanogram quantities of Ag and a detailed analysis of antimicrobial performance and size/coverage dependence thereof. This method can generate highly uniform sizes and shapes of NPs (defined as nanodots) on the surface of a substrate using techniques developed from block copolymer (BCP) patterning, since it provides a method to nanoengineer surfaces without expensive lithographic techniques.

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Block copolymers are composed of two or more chemically distinct, and usually immiscible, polymer chains which are covalently bound together (Nunns, Gwyther, & Manners, 2013). Bates and Fredrickson (1999) and Segalman (2005) reported that BCPs can self-assemble into a number of highly regular nanostructured morphologies due to the chemical incompatibility of the blocks by a process known as microphase separation. This phase behaviour can be controlled by the molecular weight, the strength of the interaction between the blocks, represented by the A-B Flory-Huggins interaction parameter, the degree of polymerization, and the volume fraction of the blocks (Farrell, Fitzgerald, Borah, Holmes, & Morris, 2009). The connectivity of the blocks in BCPs prevents complete separation and instead the diblock copolymer chains organise to put A and B portions on opposite sides of an interface (Segalman, 2005). The self-assembly of BCP has been used extensively to produce regular microstructures ranging from 5 to 50 nm in length simply by adjusting the molecular weight of the copolymer (Bang, Jeong, Ryu, Russell, & Hawker, 2009). The architecture and composition of the BCP affect the morphologies of the self-assembled structure and form phases such as spheres, cylinders, bicontinuous grids or lamellae (Bates & Fredrickson, 1999; Hamley, 2001).

It has been reported that Ag NPs have good antimicrobial activity against a wide range of bacteria, fungi, algae, and viruses (Mendes et al., 2012; Morones et al., 2005); whilst also showing low toxicity to mammalian cells (Zhao & Stevens, 1998). The antimicrobial activities of Ag NPs appear to be significantly higher than other metal NPs (silver > copper > gold > zinc > ferum) (Li et al., 2011). The inclusion of antimicrobial materials in food packaging can extend the shelf-life and preserve the quality of food by delaying/minimizing the growth of microorganisms (Appendini & Hotchkiss, 2002; Brody, Bugusu, Han, Sand, & McHugh, 2008; Kerry, O'Grady, & Hogan, 2006). Silver has been used commercially as an antimicrobial agent incorporated into plastics used as food-contact materials in Japan, the USA, and Australia (Quintavalla & Vicini, 2002; Silvestre, Duraccio, & Cimmino, 2011). However, the EU has restricted the use of Ag based on safety by limiting the amount of Ag^+ to <0.05 mg Ag/kg of the food matrix, and, although this is yet to be proven, it has been suggested, that EU consumers have a low acceptability to these products (Fernández et al., 2009; Silvestre et al., 2011). Many publications in the area of antimicrobial packaging have focused on the direct insertion of Ag NPs into a bulk polymer matrix via casting, lamination, extrusion or co-extrusion (Ghosh & Maiti, 1996; Kanmani & Rhim, 2014; Rhim, Wang, & Hong, 2013; Sánchez-Valdes, Ortega-Ortiz, Valle, MedellÍn-RodrÍguez, & Guedea-Miranda, 2009; Yeo & Jeong, 2003). These methods of insertion can be ineffective since the dispersion of the NPs can be non-homogeneous and the majority of the Ag NPs are not in direct contact with the food product, and, further, the antimicrobial effects become highly mass transport limited (Kumar, Howdle, & Münstedt, 2005). Novel methods, such as atomic layer deposition, oxygen plasma, electrochemical deposition, UV irradiation, ion implantation and sputtering (Goddard & Hotchkiss, 2007; Marini et al., 2007; Standridge, Schatz, & Hupp, 2009), can be used to attach Ag NPs to substrates, however, these techniques are expensive and need specialised equipment (Marini et al., 2007).

To date, the research on BCP patterning has primarily focused on semiconductor and electronic applications (Kim, Park, & Hinsberg, 2010), but the ability to produce ordered nano-arrangements may have widespread applicability. Block copolymer techniques can be used to place NPs (nanodots) on the surface of substrates (Huang, Kim, Tao, Connor, & Yang, 2005), however, to the best of our knowledge, BCPs have not been used as a methodology to produce antimicrobial surfaces in food-packaging and related industries. Therefore, the objectives of this study were to fabricate different sizes of mono-dispersed and well-ordered Ag nanodots using different number average molecular weight (M_n) of BCP PS-b-PEO with different concentrations of $AgNO_3$ and the manufactured materials were characterised and their potential antimicrobial properties were tested against *Pseudomonas fluorescens (P. fluorescens)*: (Gram-negative) and *Staphylococcus aureus (S. aureus)*: (Gram-positive) bacteria.

2. Materials and methods

2.1. Materials

PS-b-PEO BCPs ($M_n = 32–11$ kg mol^{-1}, $M_n = 42–11.5$ kg mol^{-1} and $M_n = 102–34$ kg mol^{-1}) were purchased from Polymer Source Inc. and used as received. $AgNO_3$ (99% min), anhydrous ethanol (99%), acetone (99%) and toluene (99%) were purchased from Sigma-Aldrich.

2.2. Preparation of polymer thin films

Microscope glass slides (size 2.5 cm × 2.5 cm) were used as a test substrate. Before use, these were ultrasonicated (Cole-Palmer 8891) for 30 min in ethanol and then acetone. The PS-b-PEO films were fabricated using a modified methodology according to Ghoshal et al. (2013). Before use, the PS-b-PEO solution (1 wt.%) was prepared in toluene and stirred in a sealed vessel for 12 h. PS-b-PEO solutions were spin-coated onto the glass slides at 3000 rpm for 30 s (Speedtime Technologies Direct View 1000 Model P6700). Microphase separation of BCP was achieved by exposing (50 °C for 1 h in a sealed vessel) as-coated films of M_n 32–11 and 102–34 PS-b-PEO to toluene, whilst a mixed vapour of toluene/water was used for M_n 42–11.5. The solvent-annealing technique was used to generate regular orientation and long-range order of the BCP thin films (Sinturel, Vayer, Morris, & Hillmyer, 2013). The glass slides containing the thin films were taken out quickly and dried under a N_2 gas stream. The films were then subjected to spin coating (3000 rpm for 30 s) with different concentrations (0.4, 0.6 and 1.2% w/v) of the Ag precursor ($AgNO_3$) dissolved in ethanol. A few sets of glass slides with single or double coatings of the Ag precursor (2 and 4% w/v) were also prepared for the optimisation process, other than to monitor the differences in terms of the morphology variation and antibacterial activity. From previous studies, a higher M_n of BCP PS-b-PEO formed a bigger cylindrical template, (Kehoe et al., 2012) and, thus, it can be filled with a higher concentration of the Ag precursor. A UV/ozone treatment (PSD Pro Series Digital UV Ozone System, UV source: 180–254 nm) was carried out for 3 h to remove any remaining solvent and organic polymer residue and the presence of ozone (O_3) acted as an oxidising agent reducing Ag^+ to Ag nanodots.

2.3. Characterisation

The surface morphologies were imaged by scanning probe microscopy (SPM, Park systems, XE-100) using the tapping mode and scanning electron microscopy (SEM, FEI Company, FEG Quanta 6700). The crystallinity of the Ag nanodots was examined by X-ray diffractometer (XRD, PANalytical MPD) using an Xcelerator detector and a Cu K_α radiation (45 kV/40 mA) between 20 and 50° 2θ. Elemental composition analysis was performed using Energy disperse X-ray spectroscopy (EDS). For AFM and SEM imaging silicon wafer substrates were used instead of

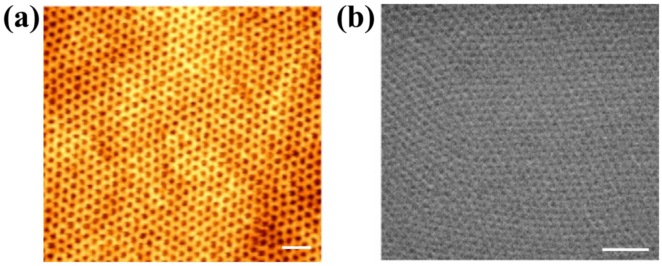

Fig. 1. AFM (a) and SEM (b) images of M_n 42–11.5 PS-b-PEO thin films annealed in mixed solvents toluene/water. Scale bar: 200 nm.