International Journal of Biological Macromolecules 91 (2016) 329–337

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

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

Covalently-layers of PVA and PAA and *in situ* formed Ag nanoparticles as versatile antimicrobial surfaces

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ARTICLE INFO

Article history:
Received 8 March 2016
Received in revised form 8 April 2016
Accepted 14 May 2016
Available online 16 May 2016

Keyword:
Polyethylene terephthalate
Ultrathin polymer layers
Antimicrobial surface
Ag nanoparticles
Solid-state grafting

ABSTRACT

The *in situ* synthesis of silver nanoparticles (AgNPs) within covalently-modified poly(ethylene terephthalate) (PET) films possessing ultra-thin layer of poly(vinyl alcohol) (PVA) and poly(acrylic acid) (PAA) is successfully demonstrated. The resulting polymeric films are shown to exhibit antimicrobial activities toward Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria and fungus (*Candida albicans*). To make the films, first PET surfaces were subject to photo-oxidation and subsequent solid-state grafting to attach a PVA layer, followed by a PAA layer. To synthesize the AgNPs inside the films, the PVA and PAA-modified PET was soaked in $AgNO_3$ solution and the polymeric film was modified with the Ag^+ ions *via* Ag^+-carboxylate interaction, and then the Ag^+ ions-containing polymer film was subject to either photo-reduction or thermal reduction processes. The PVA and PAA thin layers attached by covalent bonds to the PET surface uniquely promoted not only the *in situ* synthesis but also the stabilization of AgNPs. The formation of the AgNPs was confirmed by UV–vis spectroscopy or by monitoring the surface plasmon resonance (SPR) peak associated with AgNPs. The resulting PVA and PAA ultrathin layers modified and AgNPs containing PET served as bactericide and fungicide, inhibiting the growth of bacteria and fungi on the surfaces. Given PET's versatility and common use in many commercial processes, the method can be used for producing plastic surfaces with versatile antimicrobial and antibacterial properties.

1. Introduction

Owing to their interesting electronic, plasmonic/optical, physical, chemical and bioactive properties, silver nanoparticles (AgNPs) have long emerged as important nanomaterials for various nanotechnological applications [1–3]. By taking advantage of their properties, AgNPs have been successfully demonstrated to have numerous applications in biosensing and biodetection and as antibacterial agents against a broad spectrum of bacterial and fungal species [4–6]. In recent years, there has particularly been a burgeoning interest in AgNPs'antibacterial and antifungal uses [7,8], with some products containing them (e.g., socks) already making it into commercial uses.

Scientific reports on the properties and uses of colloidal silver against bacteria and fungi actually goes back to the late 18th century, with their applications surging between 1910 and 1920 [9]. However, with the discovery of organic compounds with antibiotic activities, such as penicillin, the use of colloidal solutions of silver began to receive less attention. The recent increase in the number and type of antibiotic resistant bacteria, such as methicillin-resistant *Staphylococcus aureus* (MRSA) worldwide [10], and the limited choices of antibiotics at our disposal to combat them, has been forcing health agencies and academics alike to look for new ways to prevent the spread of such bacteria, fungi and mutated viruses severely harming the public [11,12]. Hence, AgNPs and their broad spectra biocidal activity have once again been on the spotlight and are currently being investigated in order to combat the proliferation of various bacteria and fungi [13,14].

With the advent of synthetic tools and methods involving nanostructured materials, numerous types of Ag nanomaterials

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http://dx.doi.org/10.1016/j.ijbiomac.2016.05.056
0141-8130/

having a range of antibacterial activity toward *Escherichia coli* and *Staphylococcos aureus*, have been reported [15–17]. However, despite the well-known antimicrobial effects of Ag nanomaterials (and Ag compounds), the exact mechanism involved in Ag's antimicrobial action still remains not completely understood. Nevertheless, there are evidences indicating that the antimicrobial action of AgNPs varies on particle size and the oxidation state of Ag (bulk, ions and nanoparticles) [18].

Generally compared with organic antibacterial agents, for instance, quinolones, tetracyclines, sulfonamides and glycopeptides, inorganic antimicrobial agents have advantages due to their greater ability to prevent multi-drug resistance [19], lower toxicity and increased stability especially when applied as supported antimicrobial agents. Moreover, they are easier to apply or use [20]. In particular, materials such as AgNPs can be immobilized into polymeric matrices by letting the latter interact with the silver precursors or pre-made nanoparticles [21]. Although some polymeric materials with antibacterial properties can be easily generated this way, most engineering plastics (*i.e.*, PET, HDPE, LDPE, PS, and PVC), which otherwise have diverse applications, do not have the ability to interact with Ag species, and thereby are difficult to immobilize with AgNPs [22].

PET, in particular, is one of the most widely and commonly used engineering plastics in commercial and consumer products globally. Some examples of its uses include as food packaging material, additive in cosmetic formulations, clothing, component of medical supplies and many others. Its good stability in the presence of body fluids and its high resistance to radiation make PET a great contender material among many polymers for making biomaterials [23–25]. However, to endow PET multiple functions, especially for the use in the field of healthcare (*e.g.*, antibacterial PET fabric), placing antibacterial and antifungal agents such as AgNPs on it is desirable. With its low cost and ability to avoid the growth of pathogenic microorganisms (*e.g.*, bacteria and fungi), this kind of material (PET with AgNPs) can find applications in a variety of products for daily uses and sophisticated medical devices [26]. Recently, Deng et al. demonstrated a method to modify the surfaces of PET using atmospheric pressure plasma deposition process, which can simultaneously oxidize the PET surfaces as well as help the deposition of AgNPs on the PET's surfaces [27]. This modification process involves surface oxidation, a commonly used method to increase the surface wettability of engineering plastics and introduce functional groups that can interact with nanomaterials such as AgNPs [28–30] however, this method is complicated and has several disadvantages because it can lead to a low density of functional groups, a low selectivity to desired functional groups, less tailorable modified layer, and compromised bulk properties in the polymer when the modification is extended to a thicker surface layer. Therefore, it still remains a great challenge to synthesize and stabilize metal nanoparticles on the surfaces of polymers such as PET due to their lack of polar functional groups that can strongly bind to metals such as Ag. This subsequently leads to the leaching of Ag off of the polymeric matrix, making the material to lose its antimicrobial activity over time. So, for such materials indirect chemical modification of the polymeric substrates with groups that have the ability to interact and stabilize AgNPs could work.

In particular, covalent immobilization of ultrathin layer of polymers with functional groups that have higher capability to interact with silver can be a simpler and more innovative route to produce silver-containing polymeric surfaces than the methods previously reported in the literature. This is because the former can lead to surfaces with three dimensional space controlled thickness, to incorporate the silver nanoparticles more easily on a wider surfaces and in a more controlled manner. The preparation of ultrathin layer covalently supported in polymers substrates can be carried out for different polymer substrates, including polyethylene and polypropylene [31–34].

To this end, in the present work, we have modified PET, a very commonly used plastic material for a range of applications [35], with ultrathin layers of PVA and PAA *via* covalent attachment and then supported on it AgNPs. An important advantage of the modification method used herein is that it can easily improve the surface characteristics of the PET substrate and make it suitable for anchoring metals without altering its underlying bulk properties [31–36]. The advantage of the covalently attached thin upper polymeric layers reported here is that they produce a very stable layer to support AgNPs over the PET surfaces. Given that antibacterial activity of AgNPs is dependent not only on particle size [37], but also on their uniformity, controlling the dispersion of the NPs with the reported synthetic method allowed for the synthesis of good antibacterial and antifungal surfaces [38].

2. Materials and methods

2.1. Materials

Poly(ethylene terephthalate) (PET) (Commercial Mylar), acetone (F. Maia, ≥99.5%) and poly(vinyl alcohol) (PVA, 88% hydrolyzed and MW ranging from 13,000 to 23,000 g/mol, Sigma-Aldrich) were used as received without further purification. PAA was prepared by thermal polymerization of 5 mL acrylic acid (Acros organics, 99.5%), 50 mg of benzoyl peroxide (Vetec), and 30 mL tetrahydrofuran (THF) (Acros organics, 99%) under reflux for 2 h. The obtained PAA has an average molar weight (MW) of 15,200 g/mol). Silver nitrate ($AgNO_3$) was purchased from Merck). The microbes used in the inhibition zone test included *Escherichia coli* ATCC-25922 (Gram negative), *Staphylococcus aureus* ATCC-25923 (Gram positive) and *Candida albicans* ATCC-10231 (fungus).

2.2. Treatment PET films

The dimension of the PET films used in the studies was 3 × 4 cm with a thickness of 100 μm. The PET films were cleaned in Soxhlet using acetone for 24 h. They were then stored in a desiccator under vacuum prior to use.

2.3. PET films oxidation by UV radiation

The PET films were oxidized (on both sides) using a low-pressure mercury lamp (250 W) placed at a distance of 5 cm from the sample in a dark chamber for 1 h to subject the films to photooxidation.

2.4. Procedure for immobilization of poly(vinyl alcohol) (PVA) and poly(acrylic acid)

The UV-treated PET films above were placed in Petri dishes and covered with an aqueous solution of 5% (w/v) PVA. The Petri dishes were maintained in an oven for 24 h at 70 °C to let the solvent evaporate. Subsequently, the temperature was increased to 200 °C, and the samples were kept at this temperature for 10 min. After the heat treatment, the samples were extracted in Soxhlet for 24 h using distilled water to remove residual PVA that was not attached to the substrate. The film modified with PVA was then soaked in a solution containing 5% PAA (w/v), and the polymer solution was evaporated forming a solid PAA layer on the substrate surface. The Petri dish containing the modified PET substrate with PAA terminal layer was then subjected to a thermal treatment at 200 °C for 10 min. The film was subsequently placed in a Soxhlet extractor for 24 h to remove the PAA that was not covalently bonded to the substrate.