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Nanostructured multilayer polyelectrolyte films with silver nanoparticles as antibacterial coatings

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ABSTRACT

Ultrathin polyelectrolyte films containing silver nanoparticles appear to be a promising material for antimicrobial coatings used in the medical area. The present work is focused on the formation of multilayer polyelectrolyte films using: polyethyleneimine (PEI) as polycation, Poly(sodium 4-styrenesulfonate) (PSS) as polyanions and negatively charged silver nanoparticles (AgNPs), which led to the polyelectrolyte-silver nanocomposite coatings. The film thickness and mass were measured by ellipsometry and quartz crystal microbalance with dissipation monitoring (QCM-D) and the structure and morphology of films were visualized using scanning electron microscopy (SEM). Systematic increase of the UV–Vis absorption confirmed formation of the consecutive layers of the film. The analysis of bacteria cell adhesion to films surface was done by the luminometry measurement. Three gram-negative bacterial strains with strong adhesive properties were used in this study: *Escherichia coli*, *Aeromonas hydrophila*, *and Asaia lannenesis*. It was found that nanocomposite films have antimicrobial properties, which makes them very interesting for a number of practical applications, e.g. for the prevention of microbial colonization on treated surfaces.

1. Introduction

The development of a new generation of multifunctional coatings brings the key technology for the fabrication of functional surfaces and future high-tech products. These new coatings should combine passive functionalities inherited from "classical" ones (e.g. wettability, barrier or adhesion properties) and active functionalities that provides the response of coating properties to changes occurring either in the coating matrix (e.g. local pH change, bacteria activity) or in the local environment (temperature, humidity). From this perspective, the coatings can also have several functionalities (e.g. antiadhesive, antibacterial, antifungal, anticorrosion) exhibiting synergistic effects.

A biofouling contamination is relevant in a wide range of applications [1], e.g. for surgical equipment and protective apparels in hospitals [2], medical implants [3], biosensors [4], textiles [5], food packing [6] and storage [7], water purification systems [8] and marine and industrial equipment [9]. Surfaces that resist the non-specific adsorption of proteins and microbes are also vital for catheters, prosthetic devices, contact lenses and immunological assays [10]. The attachment of bacteria to a surface also leads to the subsequent colonization resulting in the formation of biofilm [11].

The antimicrobial properties of silver ions were known since ancient times and have been widely used as bactericide in catheters, burn wounds and dental work [12], therefore, silver nanoparticles [13–15] as well as various silver-based compounds [16,17] exhibiting antimicrobial activity have been synthesized. Silver-containing materials and coatings with antimicrobial activity can be used to reduce infections in hospitals [18–22]. Fibers containing silver nanoparticles can be utilized to eliminate microorganisms on textile fabrics [23,24]. They also exhibit a potent cytoprotective activity toward HIV infected cells [25]. Researchers have also recommended the use of silver ions as superior disinfectants for wastewater generated by hospitals containing some infectious microorganisms [26,27], however, the residual silver ions in the treated water may adversely affect human health [28]. The emergence of nanoscience and nanotechnology in the last decade presents opportunities for exploring the bactericidal effect of metal nanoparticles. It has been attributed not merely to the release of metal ions in solution. Their

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small size and high surface to volume ratio allows them interacting closely with microbial membranes [14]. However, the action of this metal is not fully known. It has been hypothesized that silver nanoparticles can cause cell lysis or inhibit cell transduction [29].

A significant number of studies have focused on surface modification by antibacterial agents. Traditional techniques involve the design of coatings with different biocidal agents, including antibiotics, quaternary ammonium salts or silver [12,30]. The universal antimicrobial surface should be environmentally neutral, does not leach and should not be consumed by microorganisms. Kim et al. [12] showed that Ag nanoparticles could be used as effective growth inhibitors in various microorganisms, therefore, have been used for antibacterial coatings, which may find application in various fields, i.e. medical instruments and devices (e.g. sensors) [31–33], water treatment and food processing. Nanoparticles may be combined with polymers to form composites allowing better utilization of their antimicrobial activity and protection from their release to the environment, since, it is also recognized that nanoparticles may have many undesirable and unforeseen effects in the ecosystem [34,35]. Therefore, their release from the composite during the whole lifecycle of a silver containing material should be controlled.

The layer-by-layer (LbL) assembly is the concept that was first introduced by Iler [36] and later applied by Decher, Hong, and co-workers [37] for preparing structure-controlled thin polymer films. Since then, the LbL technique has attracted significant interest as a simple, highly versatile approach and has been widely used to prepare nanostructured materials with tailored properties on macroscopic surfaces [38] as well as on micro- and nanoparticles [39–43]. Classically, the LbL deposition procedure involves the stepwise, electrostatic assembly of the oppositely charged polyelectrolytes, usually by consecutive dipping a substrate into the polyelectrolyte-containing solutions with the intermediate rinsing step that enables forming a multilayer coating with a nanometer scale precision. The coatings properties, such as their composition, thickness and function, can be controlled, e.g. by the number of deposition cycles, the conditions employed during the assembly process and the types of materials used. Since its introduction the LbL assembly technique has expanded rapidly to become a popular method for the preparation of various nanoscale films. It is not limited to polyelectrolyte/polyelectrolyte systems. Almost any type of nanometric charged species, including inorganic molecular clusters [44,45], nanoparticles (NPs) [46], nanotubes and nanowires [47,48], nanoplates [49,50], organic dyes [51], dendrimers [52], porphyrins [53], biological polysaccharides [54,55], polypeptides [56,57], nucleic acids and DNA [58], proteins [59–62] and viruses [63] can be successfully used as components to prepare LbL films. The driving force of LbL films formation is not limited to electrostatic interactions. Assemblies based on the hydrogen bonding [64], charge transfer [65], covalent bonding [66,67], biological recognition [68], complementary base pairing and hydrophobic interactions [69,70] have also been investigated. Besides charged inorganic substrates, hydrophobic polymer surfaces have also been shown to provide good scaffolds for LbL growth, based upon hydrophobic interactions [71]. Overall, availability of a wide spectrum of fabrication components, variety of substrates, and versatility of the assembly methods largely stimulates the biological applications of LbL films. Moreover, multiple interactions in the LbL films can potentially increase the stability of films, while being exposed to physiological or even harsher conditions.

The first report of silver nanoparticles-containing polyelectrolyte multilayers that exhibited antibacterial properties was published by Dai and Bruening [72]. They investigated the inhibition of bacterial growth by polyethyleneimine, PEI-Ag^0/poly(acrylic acid, PAA) films. They compared the growth of *Escherichia coli* in bare glass test tubes and test tubes coated with PEI-Ag^0/PAA. *E. coli*-seeded solutions in LB broth in both bare and 5.5-bilayer PEI/PAA-coated test tubes became turbid after 12 h of incubation at 37 °C. Under the same conditions, tubes coated with 5.5 bilayers of PEI-Ag^+/PAA or PEI-Ag^0/PAA ($NaBH_4$ reduced) remained clear, showing that the latter two films inhibited the growth of bacteria. Significantly, the silver nanoparticle-containing films have the same antibacterial effect as films containing silver ions. Grunlan et al. also studied the antimicrobial activity of silver ions loaded polyelectrolyte multilayers [73]. The highly effective antimicrobial thin films were prepared using layer-by-layer assembly of polyelectrolytes ((polyethyleneimine, PEI-Ag^+) or (PEI-cetrimide)/poly(acrylic acid, PAA)) in combination with antiseptic agents. Corona treatment of poly(ethylene terephthalate) PET substrates prior to thin film deposition yielded more effective films than those with no surface treatment. It is believed that the strong negative surface charge imparted by the corona led to a more concentrated deposition of the positively charged antimicrobial agents. Another useful discovery was an enhanced antimicrobial efficacy of films made with silver ions and cetrimide, an organic quaternary ammonium molecule, relative to films containing silver only. By combining two antimicrobial agents in this manner the hybrid system was created providing both strong bactericidal function in the short time scales and sustained release over longer time periods.

Antibacterial coatings based on hydrogen-bonded multilayers containing synthesized Ag nanoparticles were created on planar surfaces. The multilayers containing in situ synthesized Ag nanoparticles ((poly(allylamine hydrochloride), PAH)/(poly(acrylic acid), PAA)/(polyacrylamide, $PAAm)_nAg_x$) were formed on planar surfaces and on magnetic colloidal particles. Both Gram-positive strain (*S. epidermidis*) and Gram-negative strain (*E. coli*) bacteria were susceptible to the biocidal activity of Ag nanoparticle loaded multilayer thin films. The zone inhibition determined by the disk-diffusion test increased with the thickness of the multilayer film. The observation suggested that, in order to incrementally increase the zone of inhibition, an exponentially increasing amount of silver is required within the multilayers. The duration of sustained release of antibacterial Ag ions from these coatings, however, could be prolonged by increasing the total supply of zerovalent silver in the films via multiple loading and reduction cycles [74].

A relatively large number of techniques can be used to measure the cell adhesion. The available literature describes detection of adhered cells by rapid luminometric technique [75]. According to the literature data, this method is an efficient and reliable to determine microbial contamination of surfaces. This approach is based on bacterial ATP quantification, and it seems to be a useful tool for determination of bacterial adhesion on abiotic surfaces after antimicrobial modifications, when bacterial cells may be destroyed and they are not able to be detected by conventional plate count method [76].

Although the research on polyelectrolyte/nanosilver composite coatings have significantly advanced, challenges still remain for a fundamental exploration and their practical applications. The underlying biological mechanisms of silver nanoparticles antibacterial effect and toxicity against human cells are not fully understood yet. The research on the effect of AgNPs size, shape, and uniformity on nanosilver antibacterial ability is still in progress. The interaction of Ag nanoparticles with bacteria, the biological effect of nanosilver and polyelectrolyte/AgNPs composite coatings on bacteria adhesion and viability needs to be further investigated. Therefore, the aim of this work was to immobilize silver nanoparticles in polyelectrolyte (PE) multilayers of various thickness and to study the properties and antibacterial activity of so prepared surfaces. Multilayer films containing silver nanoparticles and poly(ethyleneimine) (PEI) were formed using the LbL method. The build-up process of PEI/AgNPs multilayer films was monitored by the quartz crystal microbalance with dissipation (QCM-D) and the UV–Vis absorption spectroscopy. The hybrid film thickness after

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