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Bacteria-responsive multilayer coatings comprising polycationic nanospheres for bacteria biofilm prevention on urinary catheters

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ABSTRACT

This work reports on the development of infection-preventive coatings on silicone urinary catheters that contain in their structure and release on demand antibacterial polycationic nanospheres. Polycationic aminocellulose conjugate was first sonochemically processed into nanospheres to improve its antibacterial potential compared to the bulk conjugate in solution (AC_{Sol}). Afterward the processed aminocellulose nanospheres (AC_{NSs}) were combined with the hyaluronic acid (HA) polyanion to build a layer-by-layer construct on silicone surfaces. Although the coating deposition was more effective when HA was coupled with AC_{Sol} than with AC_{NSs}, the AC_{NSs}-based coatings were thicker and displayed smoother surfaces due to the embedment of intact nanospheres. The antibacterial effect of AC_{NSs} multilayers was 40% higher compared to AC_{Sol} coatings. This fact was further translated into more effective prevention of *Pseudomonas aeruginosa* biofilm formation. The coatings were stable in the absence of bacteria, whereas their disassembling occurred gradually during incubation with *P. aeruginosa*, and thus eradicate the biofilm upon release of antibacterial agents. Only 5 bilayers of HA/AC_{NSs} were sufficient to prevent the biofilm formation, in contrast to the 10 bilayers of AC_{Sol} required to achieve the same effect. The antibiofilm efficiency of $(HA/AC_{NSs})_{10}$ multilayer construct built on a Foley catheter was additionally validated under dynamic conditions using a model of the catheterized bladder in which the biofilm was grown during seven days.

Statement of Significance

Antibacterial layer-by-layer coatings were fabricated on silicone that efficiently prevents *Pseudomonas aeruginosa* biofilm formation during time beyond the useful lifetime of the currently employed urinary catheters in medical practice. The coatings are composed of intact, highly antibacterial polycationic-nanospheres processed from aminated cellulose and bacteria-degrading glycosaminoglycan hyaluronic acid. The importance of incorporating nanoscale structures within bacteria-responsive surface coatings to impart durable antibacterial and self-defensive properties to the medical indwelling devices is highlighted.

1. Introduction

Indwelling urinary catheters routinely employed in clinical practice are among the medical devices that are most susceptible to microbial contaminations. Pathogens present at the site of catheter insertion or in the urine attach easily to the catheter surface and colonize it progressively to establish a mature biofilm. The bacterial cells encased in a biofilm are upto 1000 times more

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resistant to host defense mechanisms and conventional antibiotic treatments when compared to planktonic cells [1]. Once established, the biofilm serves as a reservoir that maintains the infection in the host. Consequently, their formation causes difficult-to-treat infections, unnecessary distress to patients and delays in recovery. Biofilm-associated urinary tract infections account for ~40% of all hospital-acquired infections [2], defining an urgent need for the development of novel, engineered catheter surfaces able to prevent the biofilm formation.

One approach to prevent biofilm formation on medical devices is their surface functionalization with broad-spectrum antibacterial agents. However, the substantial evolutionary stress that antimicrobials exert on bacteria ultimately results in the emergence of a multi-drug resistance species. Significant efforts are being made to design novel antibacterial surfaces limiting the bacterial colonization without promoting bacterial resistance. Existing antibiofilm materials show different efficiency toward single and multi-species biofilms and can be categorized as: (i) engineered nano- and micro-topography surfaces that inhibit bacteria adhesion [3,4], (ii) surfaces with immobilized antibiotics that kill bacteria upon adhesion [5], and (iii) biocide leaching surfaces [6,7]. All these features are imparted to the device surface using different functionalization approaches, ensuring a minimal loss of the biological functions of the deposited compound. For example, layer-by-layer (LbL) assembly of polyelectrolytes is an easy, versatile and cost-effective method for building bioactive coatings incorporating multiple modalities on a variety of surfaces. This technique is based on the alternating adsorption of oppositely charged polyions and has been used for surface immobilization of a broad range of biomacromolecules [8,9], enzymes [10], drugs [11], and nanoparticles [12], without causing structural changes that could alter their efficacy. In an attempt to engineer bioresponsive microenvironments that prevent the biofilm formation on silicone surfaces, we previously employed the LbL method to encase acylase enzyme, which quenches bacteria quorum sensing signals and interrupts their communication and the biofilm growth [13].

The incorporation of triggers in the coatings to release antibacterial agents upon external stimuli is an additional function that provides a therapeutic dose while avoiding systemic toxicity. Triggers such as pH [14], electric field [15] and temperature [16] have been frequently reported as stimuli for the release of therapeutic agents. Recently, bacteria themselves and their metabolites have been proposed as polymer hydrolyzing organisms, which can be turned into a robust trigger for antibacterial drug release in a self-defense principle [17,18]. For example, *Pseudomonas aeruginosa* (*P. aeruginosa*), a clinically relevant pathogen in most urinary tract infections, is known to produce a variety of cell-associated and extracellular metabolites including enzymes and toxins as factors for its pathogenicity [19]. One of the toxins, pyocianin, is able to degrade glycosaminoglycans (commonly used in LbL deposition) by a non-enzymatic mechanism due to its auto-oxidation properties [20]. As a redox active virulence factor, pyocianin exists in either oxidized or reduced form, the latter being an unstable free radical, which oxidizes rapidly with molecular oxygen [21]. The auto-oxidation leads to the formation of reactive oxidative species (ROS) such as superoxide (O_2^-) or hydrogen peroxide (H_2O_2), among others, capable of depolymerizing glycosaminoglycans, e.g. hyaluronic acid (HA) [22]. ROS are actually known for their oxidative degradation of different biomacromolecules (proteins, lipids and polysaccharides) [23]. The next biomedical challenge is to take advantage of such a degradation mechanism by integrating the triggers into bio-responsive coatings that will gradually release therapeutic doses of antibacterial agents and will be effective over long periods of time. LbL coatings are suitable constructs for achieving such responsiveness due to their ability to release an agent in sequential events, i.e. after the degradation of each glycosaminoglycan layer.

Cationic compounds are underutilized biocides [24] that effectively reduce bacterial count on a surface of interest without the use of antibiotics [25], thereby having great potential as antibacterial agents to reduce the risk of resistance development. Contact-killing surfaces could be easily built by LbL deposition of structures with a dense cationic charge to disrupt bacterial cell membranes and impart antimicrobial activity to a material [26]. Electrostatic binding between the cationic molecules and the intrinsically anionic bacterial cell wall induces the damage of the latter leading to cell death. This specific mechanism of action is believed to diminish the possibility of developing new resistant strains because the bacterial membrane is highly evolutionary conserved and unlikely to be changed by a single gene mutation.

Recently, we reported that cationic nanostructures in a dispersion, i.e. aminocellulose nanospheres (AC_{NSs}), possess improved antibacterial properties via a membrane disturbing capacity compared to aminocellulose in solution (AC_{sol}) [27]. In the current study, we continue the investigation by building multilayer coatings incorporating AC_{NSs} on silicone surfaces to efficiently eradicate bacteria and prevent the biofilm formation. Sonochemically-processed nanospheres obtained from the cationic conjugate, 6-deoxy-6-(ω-aminoethyl) aminocellulose, were combined with the bacteria-degradable HA polyanion to engineer LbL coatings on silicone urinary catheters and evaluate their bacteria responsiveness and antibacterial/antibiofilm capacities against the medically relevant biofilm-associated infections of *P. aeruginosa*.

2. Materials and methods

2.1. Materials and reagents

Polydimethyl/vinylmethyl siloxane Foley urinary catheters designated according to ASTM D1418 and non-shaped strips from the same material as model surfaces were provided by Degania Silicone Ltd. (Israel). Biofilm forming bacterium *P. aeruginosa* (ATCC 10145) was obtained from American Type Culture Collection (LGC Standards S.L.U, Spain). Cationic derivative of cellulose, 6-deoxy-6-(ω-aminoethyl) aminocellulose (AC, ~15 kDa), was synthesized from microcrystalline cellulose (Fluka, Avicel PH-101) via a tosyl cellulose intermediate [28], using a previously described procedure [29]. Negatively charged hyaluronic acid (HA) with average Mw ~ 750 kDa was obtained from Lifecore Biomedical (USA) in the form of its sodium salt, and used as a counterion to AC. Live/Dead BacLight kit (Molecular probes L7012) was purchased from Invitrogen, Life Technologies Corporation (Spain). All other chemical and microbiological reagents were purchased from Sigma–Aldrich unless otherwise specified.

2.2. Methods

2.2.1. Preparation of AC nanospheres

Highly cationic AC_{NSs} with sunflower oil as a lipid core were prepared by an adapted sonochemical method of Suslick [30], reported elsewhere for different kinds of biopolymers and their derivatives [31]. Briefly, the pH of the AC aqueous solution (1 mg/mL) was adjusted to 5.5 using 0.1 M HCl. Then, a mixture of 70% AC and 30% of commercial sunflower oil was prepared in a thermostated (4 °C ± 0.5 °C) sonochemical cell. The nanospheres were prepared using a Ti horn of a high-intensity Vibra-Cell VCX 750 ultrasonic processor (Sonics and Materials, Inc., USA), employing 20 kHz at 35% amplitude. The Ti horn was positioned at the aqueous-organic interface. An acoustic power of ~0.5 W/cm^3 was

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