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Air-ozonolysis to generate contact active antimicrobial surfaces: Activation of polyethylene and polystyrene followed by covalent graft of quaternary ammonium salts

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ABSTRACT

Air-ozonolysis was revealed as an accessible and effective approach for surface activation and further functionalization of hydrocarbon polymers. Antimicrobial contact active polyethylene (PE) and polystyrene (PS) were designed by generation on their surfaces OH-functional groups and covalent graft of dimethyloctadecyl [3-(trimethoxysilyl) propyl] ammonium chloride (C_{18}-TSA) quaternary ammonium salt. The shortened analog, trimethyl [3-(trimethoxysilyl) propyl] ammonium chloride (C_1-TSA), was also covalently attached to the activated PE and PS surfaces. X-ray photoelectron spectroscopy (XPS) and FTIR confirmed the surface modifications. Scanning electron (SEM) and confocal microscopy were utilized to monitor surface morphology and bacteria interactions. The antimicrobial effect of the C_{18}-TSA grafted polymer surfaces was demonstrated on Gram-negative and Gram-positive bacteria species including human pathogen, *Salmonella enterica*. The shorter C_1-TSA grafted polymers did not demonstrate bactericidal activity, suggesting the critical role of the alkyl chain length. The described strategy may establish a new general and safe platform for future development and application of contact active antimicrobial polymers.

1. Introduction

Material surfaces are most prone to bacterial colonization that causes adverse effects in various areas, resulting in contamination of medical devices, food contamination and biofouling [1]. To reduce bacterial adhesion and proliferation, contact active antimicrobial approaches were developed [2,3]. Contact active approach involves a durable (usually covalent) linkage of an antimicrobial moiety to a material's surface. Being surface linked, the antimicrobial agent is not consumed or released, providing important advantages in terms of human and environmental safety [4]. In addition, such active surfaces can be reused multiple times. Due to these environmental and operational advantages, contact active antimicrobial materials (CAAM), are of high research and applicative interest [4,5]. Contact active surface modifications were achieved through the chemical grafting of antimicrobial polymers, such as *N*-alkylated poly(4-vinylpyridine) [3], poly(4-vinyl-*N*-methylpyridinium iodide) [6], poly(butylmethacrylate)-*co*-poly(Boc-aminoethyl methacrylate) [7], covalent linkage of quaternary derivatives of acrylic acid [8] and grafting of many others antimicrobial moieties [9]. An additional domain in contact active research involves surface linkage of antimicrobial peptides [9–12].

Polyethylene (PE) and polystyrene (PS) are among the most prevalent polymers that are used in all areas of modern life, ranging from various packaging material to medicinal equipment. PE and PS have no inherent heteroatom functional groups, and therefore surface activation is required for binding of an antimicrobial moiety to these polymers. Chemical activation methods that utilize strong oxidative agents such as chromic, nitric or sulfuric acids, potassium permanganate and hydrogen peroxide can generate functional groups on the polymer surface [13]. However, such treatments produce hazardous chemical waste and may cause undesirable changes of the bulk polymer properties. Physical approaches such

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as plasma and laser treatment, corona discharge and ultraviolet (UV) radiation usually provide more precise surface modification without change to polymer bulk properties and do not involve caustic chemicals [14–16]. The only limitation of physical methods is requirement of specialized equipment, which is usually costly.

Ozone treatment is an economical and environmentally beneficial oxidative method. An oxygen balloon that provides an ozone source is required for classical ozonolysis. Ozone treatment was reported to generate carbonyl and carboxylic groups on PS and PE surfaces [17–19]. According to the best of our knowledge, no usage of the ozone-generated functional groups for the covalent linkage of antimicrobial moieties was reported. In this study, we hypothesized that ozonolysis can be an effective, accessible and safe approach for the formation of contact active antimicrobial materials. To enhance the convenience of ozone treatment, we developed an air-ozonolysis approach that does not require an oxygen balloon for ozone formation. Ozone-treated PE and PS surfaces were then modified and reacted with antimicrobial quaternary ammonium salts (TSA) to generate contact active polymers. The antimicrobial contact active TSA modified PE and PS are particularly relevant for medical devises and food packaging.

2. Experimental

2.1. Ozonolysis

The ozonolysis was performed utilizing ozonator (International Application number PCT/IL96/00090, International Publication number WO 97/0907/1) invented by Uri M. Peiper et al., at the Institute for Agricultural Engineering (ARO) and further developed by Sterilion Ltd. (Bustanai str., 18, Ramat Hasharon 47224, Israel).

2.2. Syntheses

2.2.1. Ozonolysis of PS and PE to form PS_{ox} and PE_{ox}

Additive-free polystyrene (PS) was purchased from FL Medical (Italy). The low-weight additive-free polyethylene (PE) was kindly donated by Ginegar Plastic Products Ltd. (Israel). Before usage, the PS and PE were washed three times using a Bransonic cleaner bath sonicator in ethanol (95%) and distilled water, for 10 min, each, to remove any contaminants present on the surface. Finally, the cleaned polymers were dried in a desiccator under vacuum (22 mm Hg, 25 °C) overnight.

PS and PE films (6 cm in diameter) were treated in the ozonator for 24 h at room temperature. The oxidized films were washed three times using a Bransonic cleaner bath sonicator in ethanol for 10 min and dried in a desiccator under vacuum (22 mm Hg, 25 °C) overnight.

Additive-free polystyrene (PS) was purchased from FL Medical (Italy). The low-weight additive-free polyethylene (PE) was kindly donated by Ginegar Plastic Products Ltd. (Israel). The following analytical-grade chemicals were purchased from Sigma Aldrich (St. Louis, USA) and were used without further purification: sodium borohydride ($NaBH_4$; ≥98%), lithium aluminum hydride ($LiAlH_4$; 95%), methanol (HPLC), THF (HPLC), *N*,*N*-dimethyl-*N*-[3-(trimethoxysilyl)propyl]octadecane-1-ammonium chloride (TSA_L; 72%), cetyltrimethyl-ammonium chloride (98%) and sodium fluorescein (≥98%). The *N*-trimethoxysilylpropyl-*N*,*N*,*N*-trimethylammonium chloride (TSA_S; 50% in methanol) was purchased from Gelest (USA) and was used without further purification: sulfuric acid (H_2SO_4), ethanol and water were purchased from Bio-Lab Ltd. (Israel). Water for washings was purified by the passage of deionized water through an Elgastat Spectrum reverse osmosis system (Elga Ltd., High Wycombe, United Kingdom).

2.2.2. Reduction of PS_{ox} to form PS_{red}

To a stirred solution of sodium borohydride (0.25 g, 6.25 mmol, Sigma Aldrich, St. Louis, USA) in 40 mL of water was added PS_{ox} film (0.3 g). The flask was immersed in an ice-water bath, and a solution of sulfuric acid (0.165 mL, 3.125 mmol, Bio-Lab Ltd., Israel) in water (10 mL, Bio-Lab Ltd., Israel) was added dropwise. The reaction was performed overnight with constant magnetic agitation. The film was removed from the solution, washed with water and ethanol (Bio-Lab Ltd., Israel) and then dried in a desiccator under vacuum (22 mm Hg, 25 °C) overnight.

2.2.3. Reduction of PE_{ox} to form PE_{red}

To a stirred solution of lithium aluminum hydride (0.2 g, 5.27 mmol, Sigma Aldrich, St. Louis, USA) in 50 mL of dry THF (Sigma Aldrich, St. Louis, USA), PE_{ox} film (0.2 g) was added. The reaction was performed at room temperature overnight with constant magnetic agitation. The film was removed from the solution, washed with THF and ethanol and then dried in a desiccator under vacuum (22 mm Hg, 25 °C) overnight.

2.2.4. Reaction with quaternary ammonium salts to form PS-TSA_L, PE-TSA_L, PS-TSA_S and PE-TSA_S

The PS_{red} and PE_{red} films were dipped in 30 mL of methanol (Sigma Aldrich, St. Louis, USA)/water (1:1 v/v) mixture containing 0.25 mL of TSA_L (Sigma Aldrich, St. Louis, USA) or 0.4 mL of TSA_S (Gelest, USA) at 50 °C for 24 h. Upon withdrawal from the quaternary ammonium salt solution the samples were washed three times using a Bransonic cleaner bath sonicator in methanol for 10 min and dried in a desiccator under vacuum (22 mm Hg, 25 °C) overnight.

2.3. Determination of active site density

The functionalized PS-TSA_L, PE-TSA_L, PS-TSA_S and PE-TSA_S films were incubated in a 1% solution (2 mL) of fluorescein (Na salt, Sigma Aldrich, St. Louis, USA) in water for 24 h at 30 °C. The untreated PS and PE (control) films were also treated with fluorescein to verify that there is no background absorption of the dye. Then, the films were washed with water (3 × 10 mL) using a Bransonic cleaner bath sonicator (10 min). The PE and PS films remained non-colored, while PS-TSA_L, PE-TSA_L, PS-TSA_S and PE-TSA_S became yellow. The films were placed in 2 mL of the 2.5% cetyltrimethyl-ammonium chloride (98%, Sigma Aldrich, St. Louis, USA) in distilled water and vigorously shaken for 24 h at 50 °C to desorb the dye. In this way the coordinated fluorescein was decomplexed and dissolved in the solution, and its absorption was measured using spectrophotometer (Jenway 6505 UV/Vis Spectrophotometer, Shimadzu, Tokyo, Japan). Fluorescein was excited using a 470 ± 20 nm excitation filter with a 530 ± 20 nm band pass emission filter. The absorbance of the resultant solution was measured at 499 nm. Fluorescein concentration was calculated from the Beer–Lambert equation and used to quantify the active site density of the modified materials. An independently determined extinction coefficient of fluorescein was obtained using a calibration curve and was established to be 69 mM^{-1} cm^{-1}, which is very close to the reported value [3].

2.4. Bacteriology

2.4.1. Bacterial strains and growth conditions

Bacillus subtilis PY79, *Escherichia coli* strain (ATCC 25922) and *Salmonella enterica* (SL1344) were used to test antimicrobial activity. *E. coli* strain (ATCC 25922) expressing a green fluorescent protein (GFP) was also used in this study. GFP labeling of *E. coli* strains was performed by conjugal transfer of pUC18T-mini-Tn7T-Gm-gfpmut3 into bacteria and incorporation of the *gfp* gene into the chromosomal *attB* site as described before [20]. Bacteria were maintained as glycerol stocks and stored at −80 °C. Fresh colonies