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Creation of biocidal polyethylene surface using plasma

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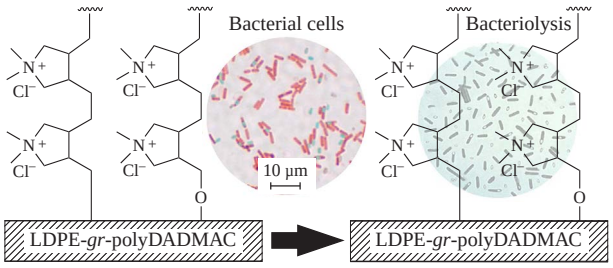
After grafting the *N*,*N*-dimethyl-*N*,*N*-diallylammonium chloride (DADMAC) polymer to low-density polyethylene (LDPE) using plasma, the polyethylene surface acquired wettability with water. The synthesized graft copolymer turned out to be bactericidal against gram-positive *Staphylococcus aureus* and gram-negative *Escherichia coli*.



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The surfaces of various materials are a growth medium for many microorganisms, often forming biofilms on them.[1–4] The growth of pathogenic microorganisms on the surface of medical and food equipment can cause a surge in infectious diseases.[5–8] In other industries, biofilms on equipment surfaces can also lead to poor performance or even destruction of equipment. The formation of biofilms is also possible on polymeric materials.[9–12] This phenomenon is most dangerous in enclosed spaces where people stay for a long time, for example, in the Russian segment of the International Space Station.[13–15] One of the ways to prevent the growth of biofilms on polymer surfaces is to create materials with biocidal properties.[16–18] It can also be cost-effective to impart biocidal properties to finished polymer products by applying substances with antimicrobial properties to their surfaces.[19,20] In this case, a more effective method is not simply applying biocidal substances (or compositions) to the surface,[16,17,21] but their strong covalent chemical attachment.[22–24] This method is most effective, since there will be no chance of removing the surface antimicrobial layer when treating with solvents or surfactants, as well as when wiping. Reactive polymer quaternary salts with a broad antimicrobial spectrum of action can be used as biocidal substances suitable for application to surfaces.[24,25] In particular, the most attractive of this class of compounds is the polymer of *N*,*N*-dimethyl-*N*,*N*-diallylammonium chloride (DADMAC), on the basis of which the disinfectant 'Septopol' has been developed and approved for use.[26] The original DADMAC monomer is produced industrially in Russia (BSC 'Bashkir Soda Company', Sterlitamak), which makes it especially convenient for use.

The quaternary salt of polyDADMAC is incorporated into the membranes of microbial cells, changes their lipid composition, inhibits the activity of proteins (H^+-ATPase) and disrupts the transport of substances into the cell.[27] In addition, it is known that polyDADMAC exhibits its activity not only in solutions, but also in the form of a polymer film on the surface.[19]

In this work, a method for immobilizing the biocide polyDADMAC on a plasma-activated low-density polyethylene (LDPE) film with a thickness of 200 μm was proposed.†

Plasma treatment of LDPE leads to the rupture of covalent C–H bonds and the formation of primary radicals.[30] Some of the radicals interact with active oxygen in the plasma to form oxygen-containing groups on the polymer surface. When the film is unloaded from the reactor, long-lived radicals interact with atmospheric oxygen, forming peroxide groups.[22,30,31] When immersing the LDPE film in a DADMAC solution, these peroxide groups initiate radical graft polymerization of reactive DADMAC to form a grafted polymer with a cyclolinear structure.[32]

The appearance of a polyDADMAC layer on the hydrophobic surface of LDPE is evidenced by its wettability with water and aqueous solutions. Using the ImageJ program, a digital image of a drop (recorded with an HD camera) showed a decrease in the contact angles of the plasma-treated and grafted LDPE (Table 1 and Figure 1). An increase in the direct current generating the plasma (from 50 to 80 mA) also led to a decrease in the contact angles of wetting of the treated LDPE samples with water and a DADMAC solution, both with and without grafting (see Table 1).

† The process was carried out by treating the cleaned LDPE surface with O_2 plasma for 5 min (DC glow discharge 20–110 mA, gas pressure 100 Pa). The irradiated sample was immersed in a 15% DADMAC solution for 1 h to carry out the monomer grafting reaction. During the radical DADMAC grafting reaction on the LDPE surface, DADMAC homopolymer may form in the reaction system.[28,29] To exclude the influence of DADMAC homopolymer and, possibly, monomer residues on the antimicrobial properties of the modified LDPE samples, it is necessary to thoroughly remove possible impurities of the DADMAC homopolymer and monomer. It was considered insufficient to wash the modified samples with water for only 30 min, as was done, for example, in the published work,[22] so the number of washes was increased. For this purpose, the samples were soaked three times in distilled water, thoroughly washed and dried.

Table 1 Contact angles of the surfaces of the studied samples.

Sample	Contact angle/deg	
	Water	30% DADMAC solution
Original LDPE	77 ± 4	72 ± 4
LDPE + plasma (50 mA)	44 ± 6	43 ± 2
LDPE + plasma (80 mA)	42 ± 4	36 ± 2
LDPE + plasma (50 mA) + grafted polyDADMAC	33 ± 3	<20
LDPE + plasma (80 mA) + grafted polyDADMAC	24 ± 2	13 ± 1

Figure 1 Images of water droplets on the surfaces of (*a*) original LDPE, (*b*) LDPE after O_2 plasma treatment and (*c*) LDPE grafted with polyDADMAC.

This is explained by the fact that with an increase in the discharge current, the flow of active plasma particles onto the sample increases, which increases the concentration of radical centers and oxygen-containing groups on the polymer surface. This, in turn, leads to an increase in the hydrophilicity of the sample and a decrease in the contact angles. For water, the decrease in the contact angle was to a lesser extent than for the DADMAC solution, which is apparently due to the different surface tension of the liquids.

The maximum decrease in the value of water contact angles was observed in LDPE samples grafted with polyDADMAC and pretreated with higher-power plasma [see Table 1 and Figure 1(*c*)]. This is explained by the appearance of a large number of peroxide groups on the entire surface, and then hydrophilic fragments due to the grafting of long polyDADMAC chains containing hydrophilic quaternary ammonium fragments in each monomer unit. In principle, the entire surface of the modified LDPE cannot be completely covered with the generated radicals and then grafted with polyDADMAC polymer chains. Even in the absence of a continuous coating of the substrate with grafted chains, the remaining less hydrophilized areas of the substrate will be covered with long flexible hydrophilic polyDADMAC chains. A similar increase in the efficiency of acrylic acid grafting to the surface of polyethylene terephthalate with an increase in plasma power was previously observed.[33]

The study of the ATR-IR spectra of the original and grafted LDPE showed their similarity. Differences were found only in the regions of 1600–1850 and 3000–3600 cm^{-1}.

Peaks at 1630 and 1750 cm^{-1} apparently correspond to esters and amides (Figure 2). The appearance of ester and amide groups is explained by the use of traditional processing additives such as slip agents, antistatic agents, hindered amine light stabilizers or their combinations, for example, oleamide or erucamide.[34,35] Bands at 1680–1710 cm^{-1} are characteristic of carboxylic acids[36] formed

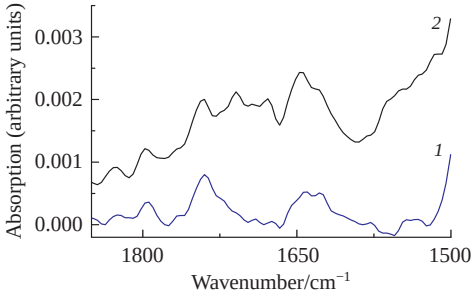

Figure 2 ATR-IR spectra of (*1*) LDPE and (*2*) LDPE-*graft*-polyDADMAC.

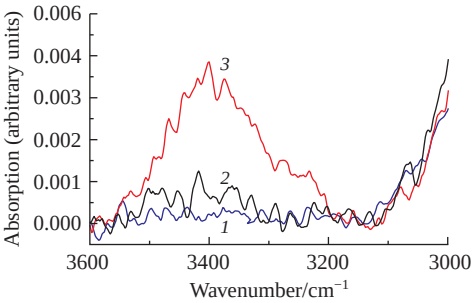

Figure 3 ATR-IR spectra of (*1*) LDPE, (*2*) plasma-treated LDPE and (*3*) LDPE-*graft*-polyDADMAC.

by surface reactions of the material during oxygen plasma treatment. The band at 1560 cm^{-1}, broader for the grafted sample, corresponds to carboxylic acid salts, including quaternary ammonium salts.[37]

In the region of adsorbed water and hydrogen bonds at 3000–3600 cm^{-1}, a broad peak is observed in the spectrum of the grafted polymer even after drying. This indicates significant hydrophilization of the surface (Figure 3). In the case of plasma treatment of the polymer without subsequent grafting, only weak peaks of single (non-hydrogen-bonded) –OH groups at 3420 and 3360 cm^{-1} are observed in the spectrum, which are absent in the original polyethylene.

Thus, IR spectroscopy confirms the grafting of polyDADMAC onto the surface of LDPE films.

The thickness of the grafted polyDADMAC layer was estimated to be 15–20 nm based on AFM results for cross-sectional profiles in several projections. The fraction of the surface covered by the grafted polyDADMAC layer was determined from digital AFM images using the Gwyddion 2.66 software. The obtained values were ~70% for a discharge current of 50 mA and ~90% for a discharge current of 80 mA from the LDPE surface, depending on the selected area. One would expect an increase in the degree of surface coverage with the grafted polymer at a higher discharge current, however, thermal degradation of the polymer is observed at currents above 80 mA. Surface activation at currents below 50 mA will be insufficiently effective due to a smaller number of active radical centers formed on the sample surface.

Thus, the thickness of the grafted layer is 25–200 times smaller than the size of the bacteria selected for the study, namely, *Staphylococcus aureus* (diameter 0.5–1.5 μm) and rod-shaped *Escherichia coli* (size 0.4–0.8 × 1–3 μm).

Unexpectedly, an attempt to determine the percentage content of grafted polyDADMAC on the LDPE film did not yield any results, since the total mass (60 + 40 = 100 mg) of two identical in area (30 × 30 mm) LDPE samples grafted with polyDADMAC turned out to be less (!) than the mass of the original unmodified sample (105 mg) of the same area. This is due to two reasons: the first is a very small specific surface area compared to fibers or dispersions and, accordingly, a small mass of the grafted polymer, and the second is the uneven thickness of the industrial LDPE film (wedge-shaped).

Table 2 Dependence of the number of surviving microbial cells on the duration of their incubation on control and modified LDPE films.

Incubation time/min	Number (percentage) of surviving cells			
	E. coli		*S. aureus*	
	Control LDPE	Modified LDPE	Control LDPE	Modified LDPE
0	982 ± 56 (100)	982 ± 56 (100)	1120 ± 68 (100)	1120 ± 68 (100)
10	911 ± 49 (92.8)	291 ± 21 (29.6)	1076 ± 84 (96.1)	243 ± 20 (21.7)
30	674 ± 31 (68.6)	63 ± 13 (6.4)	991 ± 73 (88.5)	58 ± 9 (5.2)

To evaluate the antimicrobial activity of modified LDPE films, test microorganisms from the collection of the Federal Research Center for Biotechnology of the Russian Academy of Sciences were used: gram-negative bacteria *E. coli* MG 1655 K12 and gram-positive bacteria *S. aureus* 209P, which are analogues of pathogenic strains.‡

A comparison of the number of surviving cells after incubation on the control and modified LDPE films showed the presence of a biocidal effect against both gram-negative and gram-positive bacteria (Table 2). After just 10 min of incubation on the modified LDPE film, the number of viable *E. coli* cells decreased by 70%, and that of *S. aureus* by almost 80%. On the contrary, after washing off the control samples, more than 90% of the bacteria remained viable. Increasing the incubation time to 30 min led to the death of almost 95% of the cells, indicating a strong biocidal effect of the modified LDPE. The results of this work are consistent with the data obtained in the study of modified LDPE films, on which the DADMAC biocide was immobilized by post-irradiation grafting using X-ray radiation.[38]

Thus, on the surface of LDPE, plasma generates radicals that are transformed into peroxide groups by interaction with oxygen. These peroxide groups initiate radical graft polymerization of DADMAC, forming a surface layer of polyDADMAC covalently bound to LDPE. Due to the antimicrobial film of polyDADMAC on the surface of the substrate, the resulting material is capable of killing both gram-negative and gram-positive bacteria.

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‡ A suspension of test microorganisms (100 μl) containing about 10^3 bacterial cells was applied to the surface of the modified and control films with an area of 1 cm^2. After incubation for 10 and 30 min, the cell suspension was washed off the films onto a Petri dish with L-agar medium. The Petri dish was incubated for 3 days at 28 °C, after which the number of grown colonies corresponding to the number of surviving cells was counted. The obtained data were compared with the initial number of cells applied to the films.[38] Each experiment was repeated 5 times, with the standard deviation calculated.

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