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MINI-REVIEW

Antimicrobial polymers: mechanism of action, factors of activity, and applications

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Abstract Complex epidemiological situation, nosocomial infections, microbial contamination, and infection risks in hospital and dental equipment have led to an ever-growing need for prevention of microbial infection in these various areas. Macromolecular systems, due to their properties, allow one to efficiently use them in various fields, including the creation of polymers with the antimicrobial activity. In the past decade, the intensive development of a large class of antimicrobial macromolecular systems, polymers, and copolymers, either quaternized or functionalized with bioactive groups, has been continued, and they have been successfully used as biocides. Various permanent microbicidal surfaces with non-leaching polymer antimicrobial coatings have been designed. Along with these trends, new moderately hydrophobic polymer structures have been synthesized and studied, which contain protonated primary or secondary/tertiary amine groups that exhibited rather high antimicrobial activity, often unlike their quaternary analogues. This mini-review briefly highlights and summarizes the results of studies during the past decade and especially in recent years, which concern the mechanism of action of different antimicrobial polymers and non-leaching microbicidal surfaces, and factors influencing their activity and toxicity, as well as major applications of antimicrobial polymers.

Keywords Bacteria · Antimicrobial activity · Quaternary/non-quaternary polymers · Mechanism of action

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Introduction

During the last two decades, the field of macromolecules with antimicrobial properties, including the synthesis of novel structures and modifications of known polymers, as well as biological, physicochemical, and biochemical research and engineering design, made a great advance. On the one hand, this is due to the rather complex epidemiological situation, nosocomial infections, microbial contamination, and infection risks in hospital and dental equipment, e.g., surgical armaments and fabrics, catheters, endoscope and medical implants, etc., in water purification system, food, and general consumer markets that has led to an ever-growing need for prevention of microbial infection in these various areas (McDonnel and Russell 1999; Block 2001; Gilbert and Moore 2005). On the other hand, in the 1980s, the intensive development of macromolecular science created a base for preparing various polymer structures and identified a fundamental difference between their properties and those of low molecular weight compounds, allowing efficient use of polymers in various fields, including the creation of macromolecular systems with antimicrobial activity. Advances in the methods of investigation of macromolecules such as DLS, atomic force microscopy (AFM), transmission electron microscopy (TEM), fluorescence microscopy, calorimetry (isothermal titration calorimetry, differential scanning calorimetry), etc. also contributed to the new data on the mechanism of antimicrobial polymers interactions with bacterial cells and model phospholipid membranes.

In the 1990s, antimicrobial polymer systems were surveyed by Worley and Sun (1996) and Afinogenov and Panarin (1993). A variety of antimicrobial polymers have been considered in several comprehensive reviews during the past decade. Tashiro has reviewed processes of

synthesis of antimicrobial polymers such as polyionenes (polymers with positively charged nitrogen atoms located in the backbone of a macrochain), cationic macromolecules containing pendant positively charged active groups including biguanide, quaternary ammonium salts (QAS), and quaternary pyridinium or phosphonium salts, as well as studies on their antibacterial activity (2001). The review by Kenawy and co-authors was focused on the chemical variety of antimicrobial macromolecular systems, different approaches to the preparation of antimicrobial polymers or copolymers (synthesis of novel monomers, attaching bioactive substrates such as quaternary ammonium or phosphonium groups, and some others, and cyclic *N*-halamine compounds to synthetic monomers or polymers, modification of naturally occurring macromolecules), as well as on antimicrobial activities of the polymers and also major applications of antimicrobial macromolecular systems (2007). The review by Tew and co-authors has covered antimicrobial polymers whose biological activities are influenced by the amphiphilicity of the polymer or oligomer as a whole rather than the activity of an antimicrobial moiety either embedded or covalently attached, and included mainly antimicrobial macromolecules that can mimic the biological activity of the antimicrobial natural host-defense peptides (HDPs) [synthetic poly(phenylene ethynylenes), polynorbornenes, polymethacrylates], as well as membrane perturbation and biophysical techniques, and application in materials (Gabriel et al. 2007). The review by DeGrado and co-authors was focused on the antimicrobial monomers and polymers that can mimic antimicrobial HDPs (Tew et al. 2010).

The present mini-review will briefly highlight the results of studies during the past decade and especially in recent years subsequent to the aforementioned reviews, which concern the mechanism of action of different antimicrobial polymers and factors influencing their activity and toxicity. Hereupon, some applications of antimicrobial polymers will be discussed.

Mechanism of action and factors of activity

The main strategy for designing synthetic antimicrobial polymers has been determined by the common structural features of the outer envelope of different bacterial cells. The important characteristic of the outer envelope of the cells is a net negative charge (often stabilized by the presence of divalent cations such as Mg^{2+} and Ca^{2+}). It is provided by the teichoic (or lipoteichoic) acid molecules of Gram-positive bacteria cell wall (CW), the lipopolysaccharides and phospholipids of Gram-negative bacteria outer membrane (OM), and the cytoplasmic membrane (CM) itself, which is composed of a phospholipid bilayer with embedded essential functional proteins, such as enzymes. The cytoplasmic membrane has selective permeability properties (it is semi-permeable) and regulates the transfer of solutes and metabolites in and out of the cell cytoplasm (Singer and Nicholson 1972; McDonnel and Russell 1999; Maillard 2002; Franklin and Snow 2005; Gilbert and Moore 2005). Based on the features of the CW/OM and CM of a cell, the major part of antimicrobial polymers was designed as cationic hydrophilic–hydrophobic macromolecular systems, a target site for which the cytoplasmic membrane was considered (so-called membrane active agents). There are polymers with links containing a hydrophilic polar functional block bearing cationic charge and a hydrocarbon non-polar hydrophobic block (or hydrophobic structure of a whole link), or random copolymers formed by a hydrophobic monomer and a hydrophilic comonomer with a functional group. Such polymer/copolymer structures provide surface-activity properties and adsorption/absorption ability (so-called surfactants), and high binding affinity for bacterial cells enhanced by high lipophilicity in order to cause effective damage of the structural organization and integrity of cell membranes, followed by CM disruption (in the major part of cases), leakage of cytoplasmic contents, and cell lysis (Denyer and Stewart 1998; Hugo 1999; Merianos 2001; Tashiro 2001; Kenawy et al. 2007; Gabriel et al. 2007).

Mode of action

Common cationic polyelectrolyte salt polyhexamethylene biguanide chloride (PHMB) (Fig. 1) was the first antimicrobial polymer whose mechanism of interaction with *Escherichia coli* and phospholipid membranes (modeling bilayer membrane of a cell) was studied by Gilbert and coworkers (Broxton et al. 1983, 1984) and Ikeda et al. (1983, 1984a). PHMB was shown to cause domain formation of the acidic phospholipids of the CM in the vicinity of the adsorption site (Ikeda et al. 1983, 1984a) and to impair the integrity of the OM of Gram-negative bacteria *E. coli* (Broxton et al. 1984). Importantly, the extent of membrane disruption was shown to increase with the growth of polymer length (from two links to greater than ten) (Broxton et al. 1983). The sequence of events during PHMB interaction with the cell envelope of *E. coli* was proposed as follows: (1) there is rapid attraction of PHMB toward the negatively charged bacterial cell surface, with strong and specific adsorption to phosphate-containing

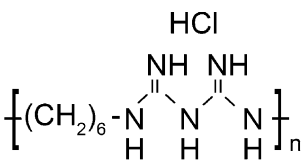


Fig. 1 Poly(hexamethylene biguanide chloride) (PHMB)

compounds; (2) the integrity of the outer membrane is impaired, and PHMB is attracted to the inner membrane; (3) binding of PHMB to phospholipids occurs, with an increase in inner membrane permeability (K^+ loss) accompanied by bacteriostasis; and (4) complete loss of membrane function follows, with precipitation of intracellular constituents and a bactericidal effect (Franklin and Snow 1981; Broxton et al. 1983, 1984; Ikeda et al. 1983, 1984a; McDonnel and Russell 1999; Maillard 2002; Gilbert and Moore 2005).

Quaternary ammonium/phosphonium polymers

In the 1980s, Ikeda et al. have synthesized two novel cationic polyelectrolytes, polymethacrylate containing pendant biguanide groups and polyvinylbenzyl ammonium chloride (Fig. 2), and showed their high biocidal activity against *Staphylococcus aureus* and *E. coli*, which was much higher than that of the monomeric species (Table 1) (Ikeda et al. 1984b, c). Reported data on the lysis of protoplasts of *Bacillus subtilis* in contact with the polycations have shown that the target sites of the quaternary polymers are the CM of bacteria (Ikeda et al. 1986).

The results of investigations carried through the last decade with different experimental methods, such as Live/Dead two-color fluorescence, TEM, AFM, monitoring of loss of bacterial cells constituent, and/or experiments on polymer-induced dye leakage from model liposomes, support the hypothesis that antimicrobial polymers bearing cationic charge on the quaternary ammonium/phosphonium groups kill bacteria by damaging the OM/CW and cytoplasmic membrane, followed by cell lysis that has been observed in solution (Tashiro 2001; Grapski and Cooper 2001; Chen and Cooper 2002; Tew et al. 2002; Kenawy and Mahmoud 2003; Kenawy et al. 2007; Waschinski et al. 2008b; Rawlinson et al. 2010) and on surfaces (Lee et al. 2004; Hu et al. 2005; Milovic et al. 2005; Park et al. 2006). For instance, Kenawy and Mahmoud have synthesized copolymers of 2-chloroethylvinyl ether and vinylbenzylchloride with immobilized ammonium or phosphonium salts (Fig. 3a) and, using electron microscopy, demonstrated that one of the most effective phosphonium-containing cationic polymers causes the CM disruption of *S. aureus* cells and a release of potassium ions, as it was shown by the assay of potassium leakage (Kenawy and Mahmoud 2003).

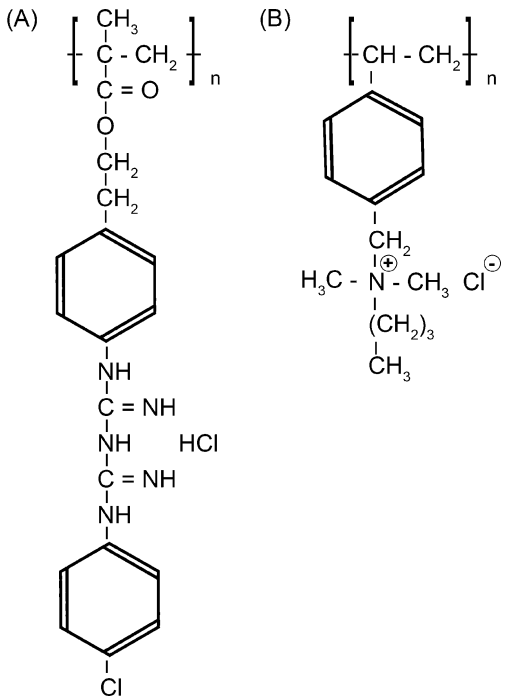


Fig. 2 **a** Polymethacrylate containing pendant biguanide groups. **b** Poly(vinylbenzyl ammonium chloride)

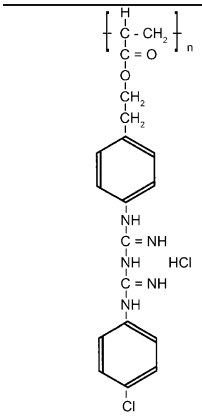
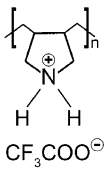
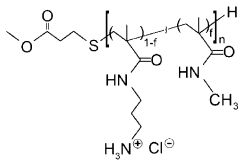
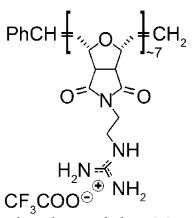
N-halamine polymeric compounds

There is a large class of biocidal polymers and copolymers, cyclic *N*-halamine polymeric compounds, whose mechanism of action is distinct from the aforesaid of membrane active antimicrobial polymers. *N*-halamines and later polymers with *N*-halamine functional groups have been developed by Sun, Worley, and co-workers to stabilize the antimicrobial properties of free halogens (chlorine or bromine) (Worley and Sun 1996; Kenawy et al. 2007) (see 'Application' section). In *N*-halamines, one or more halogen atoms are covalently bonded to the nitrogen atoms of the compounds which provide stability and slowly release free active halogen species into the environment. The main biocidal impact of the *N*-halamines relates to a specific action of oxidative halogen (Cl^+ or Br^+) targeted at a biological receptor (thiol groups or amino groups in proteins) upon direct contact with a cell, leading to cell inhibition or cell inactivation, rather than polymer action itself, for instance polymeric quaternary ammonium salt with a long alkyl radical (Worley and Sun 1996; Denyer and Stewart 1998; Makal et al. 2006; Shirai et al. 2006; Kocer et al. 2008; Kou et al. 2009).

Polymers with bioactive substrates

One of the approaches to increase the efficiency of antimicrobial polymer systems while lowering their potential toxicity is attaching bioactive substrates to macromolecules that was discussed by Kenawy et al. (2007). An example of immobilization of the antimicrobial agent on synthesized polymer is the investigation by Kenawy and co-workers on attaching different functional groups to polyacrylamide (Kenawy et al. 2006). Polyacrylamide modified by introducing an amino group in the side chain of the polymer was

Table 1 Antimicrobial activity of different synthetic cationic polymers in solutions

Polymer structure	M_w/M_n (kDa); PDI; DP [a]	Microorganism	Micro-organism concentration, cells/mL; time of contact	Minimum inhibitory bactericidal concentration (MIC), μg/mL; selectivity [b]	Ref.
	M_w 11.9[c]	*E. coli, S. aureus*	5×10^4; 30 min	1.0 (*S. aureus*); 40 (*E. coli*)	Ikeda et al. 1984
	M_w 62; PDI=1.85;[d] DP=160	*E. coli, S. aureus, Candidaa lbicans, Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella pneumoniae*	5×10^7; 1.5h	1.5 (*S. aureus, C. albicans*); 13–17 (*E. coli, K. pneumoniae*); 28–34 (*P. mirabilis*); 118–132 (*P. aeruginosa*)	Timofeeva et al. 2009
	M_w 24; PDI=1.85;[d] DP=62	*E. coli, S. aureus, Candidaa lbicans*		28–34 (*S. aureus*); 3.5 (*C. albicans*); 118-132 (*E. coli*)	
	M_n 1.0 DP=6.2; f=0.47[e]	*E. coli*	~2×10^5; 18h	16; Sel > 25	Palermo and Kuroda 2009
	M_n 1.1; DP=6.2; f=0.18[e]			1,000; Sel > 2	
	M_n 2.5[f]	*E. coli, S. aureus, B. subtilis,, Serratia marcescens*	~10^5; 6 h	MIC_{90}[g] 6 (*E. coli*); 12 (*S. aureus, B. subtilis*) 50 (*S. marcescens*) Sel = 250[g]	Gabriel et al. 2008

[a] Explanations to molecular weights M_w, M_n see in the text; coefficient of polydispersity PDI = M_w/M_n; DP is a degree of polymerization (i.e., an average number of links; see also text)

[b] Where it is not pointed especially, there is minimum inhibitory bactericidal concentration killing 100% of bacteria (MIC_{100}). Selectivity, Sel = HC_{50}/MIC, where HC_{50} is the hemolytic concentration lysing 50% of blood cells

[c] M_w was determined by LS

[d] M_w was determined by ultracentrifugation; M_n and PDI were determined from ^{1}H NMR spectra

[e] Parameters were determined from ^{1}H NMR spectra

[f] M_n was determined by GPC

[g] Minimum inhibitory bactericidal concentration killing >90% of bacteria (MIC_{90}); selectivity is estimated for *E. coli*

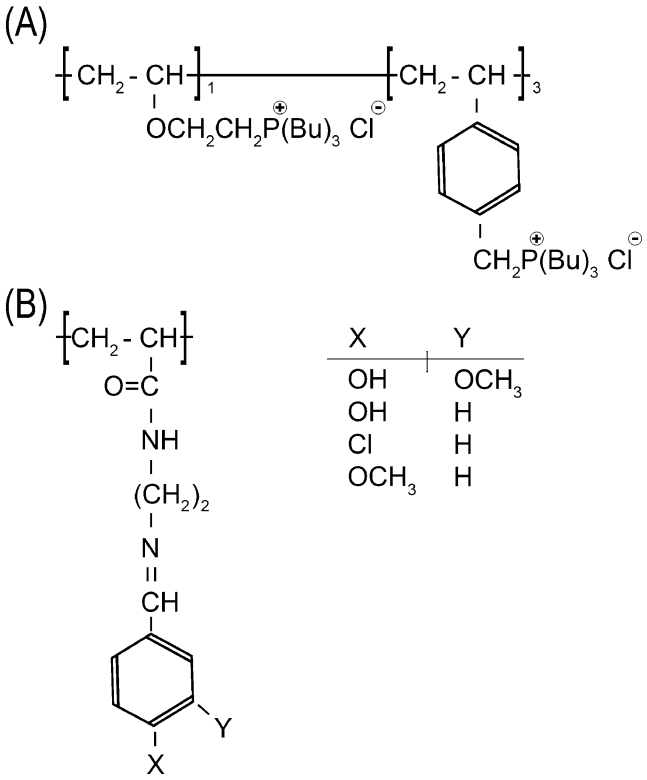


Fig. 3 **a** Copolymer of 2-chloroethylvinyl ether and vinylbenzylchloride with immobilized tributylphosphonium salt. **b** Amine-modified polyacrylamide with immobilized aromatic aldehydes containing active groups such as *p*-hydroxybenzaldehyde, vanillin, *p*-chlorobenzaldehyde, and anisaldehyde

functionalized with aromatic aldehydes containing active groups, or phenolic ester derivatives, where the polymer derivative of *p*-chlorobenzaldehyde has been revealed to be the most effective against a representative set of bacteria and fungi species (Fig. 3b) (Kenawy et al. 2006). It has been stressed that the mode of action, in particular for phenolic ester derivatives, is related to the phenolic moieties rather than the polymer itself (Kenawy et al. 2006).

Molecular mechanism of antimicrobial action

To understand the organization of interactions between polycations (including non-biocidal) and cell membranes, model lipid bilayer membranes were used, which simulate the permeability barrier of cell membranes. Numerous studies of interactions between linear cationic polyelectrolytes, such as ammonium polybases [polylysine, polyallylamine, poly(ethylenimine) (PEI)] and quaternary ammonium polysalts [polyionenes, quaternized poly(vinylpyridine) (PVP)], and model lipid membranes have revealed common features. These are (1) formation of interface complexes between the membrane and the polycation that are stabilized by multiple ion contacts of free polycation ammonium links (i.e., unscreened by counterions) with negatively charged groups of lipid molecules and membrane proteins, and (2) translocation of the negatively charged molecules of lipid from the inside leaflet to the outside leaflet of the membrane (effect "flip-flop") and lateral segregation of the negatively charged lipids (Carrier et al. 1985; Oku et al. 1986; Ikeda et al. 1990; Franzin and Macdonald 2001; Yaroslavov et al. 2002, 2006).

Structural parameters of polymers

Since the works by Ikeda et al. in the 1980s, various cationic polyelectrolytes, amphiphilic polymers bearing cationic functional groups, and also copolymers exhibiting antimicrobial properties in solutions and on surfaces have been synthesized (Tashiro 2001; Kenawy et al. 2007; Gabriel et al. 2007). These and other investigations including studies of the past years shed light on some issues regarding the general features of the mechanism of different cationic polymers action and the factors affecting the antimicrobial properties and, at the same time, hemolytic/cytotoxic activities. The factors include such parameters of macromolecular system as molecular weight (MW), polycation charge density as well as a type of counterion, the overall hydrophobicity (alkyl substituents, side/end groups, or architecture of a polymeric link), and hydrophilic–hydrophobic balance.

Molecular weight effect

Molecular weight of polymers (i.e., average number of links or degree of polymerization, DP) as well as their alkylation has been shown to profoundly impact the efficacy of many antimicrobial quaternized macromolecular systems. Ikeda et al. have found that the activities of the synthesized polycations are strongly dependent on their MW and have a bell-like shape, thus, an optimal MW range (the weight-average molecular weight, M_w, from 50 to no more than ~100 kDa) exists for the cidal action of the polymeric biocides (Ikeda et al. 1986). At the same time, an experiment on the lysis of protoplasts of *B. subtilis* in contact with the polycations has shown that the biocidal effect enhances with the growth of M_w. Summarizing the data, Ikeda et al. have indicated that adsorption ability and capability to penetrate through OM/CW are the primary factors that control antimicrobial activity of polycations (Ikeda et al. 1986).

The dependence of the antimicrobial activity on MW of polymers (i.e., DP) has been reported in many further publications (Tashiro 2001; Merianos 2001; Kenawy et al. 2007). Below, several examples of the studies that appeared during the past decade are listed.

Cooper and co-workers have discovered a parabolic dependence on MW of the antimicrobial properties of the synthesized quaternary ammonium functionalized poly(propyleneimine) dendrimers (Chen et al. 2000). Hönig and co-workers have prepared a series of different oligomeric

guanidines and showed that lower MWs result in a rapid decrease in activity (Albert et al. 2003). Klibanov and co-workers have revealed that quaternized and alkylated common polymers, *N*-hexyl-PVP and *N*-hexyl,*N*-methyl-PEI, when immobilized on a surface, both required a minimal polymer size to exert the full bactericidal effect: M_w 160 but not 60 kDa for PVP and 25 but not 2 kDa for PEI[1] (Fig. 4) (Table 2) (Tiller et al. 2001; Lin et al. 2003; Klibanov 2007) (see 'Antimicrobial surfaces' section). Matyjaszewski, Russell, and co-workers have demonstrated the effect of the MW growth of the synthesized poly(2-(dimethylamino)ethyl methacrylate) (PDMAEMA) on the antimicrobial efficacy of a surface grafted with the quaternized PDMAEMA: polymers of relatively high number-average molecular weight, M_n> 10 kDa, exhibited almost 100% killing efficiency, while oligomers (M_n=1.5 kDa) exerted less activity with the same grafting density (Fig. 5) (Table 2) (Huang et al. 2007) (see 'Antimicrobial surfaces' section). Timofeeva and colleagues, when exploring bactericidal activity of aqueous solutions of the novel synthesized cationic polymers secondary and tertiary polydiallylamines (PDAAs) (Fig. 6a) against Gram-positive and Gram-negative bacteria, have found that minimum inhibitory bactericidal concentration killing 100% of bacteria (MIC_{100}) of PDAAs decreases by an order of magnitude with the M_w growth from 24 to 55–62 kDa, while fungicidal activity of these polymers was not influenced by the MW remaining quite high (Table 1) (Timofeeva et al. 2009) (see 'Amine structure' section).

It is reasonable to relate the substantial boost of bactericidal efficiency of a polycation with the growth of its polymerization degree or in comparison with a monomer to such evident factors as an enhancement of hydrophobic mass, an enlargement of the polymer coil, and an increase in the net charge and absolute number of active cationic centers on a single polyelectrolyte molecule (that generally does not mean an increase in a charge density of the polycation, or a fraction of the charged polymer links), i.e., to the known cooperative properties of polymer molecules, which strengthen their adsorption ability, binding affinity, and destructive interaction with a cell of microbe. At the same time, Ikeda et al. have showed that the region of MW with high cidal activity is

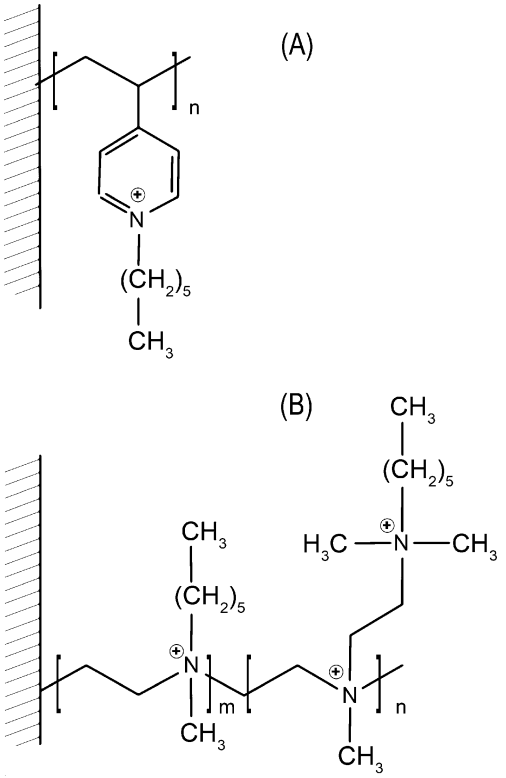


Fig. 4 Schematic representation of polymers covalently immobilized on a surface. **a** Poly(4-vinyl-*N*-hexyl-pyridinium) (*N*-hexyl-PVP). **b** Branched *N*-hexyl,*N*-methyl-poly(ethyleneimine) (*N*-hexyl,*N*-methyl-PEI)

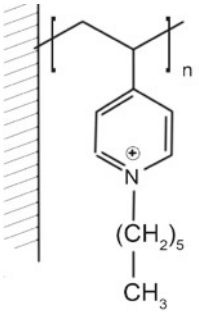
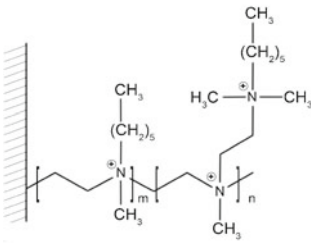
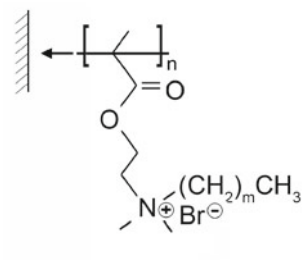
restricted from the top and related this to a decrease in the penetration capability through OM/CW with the increase in MW larger than a "critical" value (~100 kDa for the investigated polymers) (Ikeda et al. 1986). Franklin and Snow noted that the structures of CW/OM of bacteria and fungi are mainly open networks of macromolecules and usually do not offer an efficient barrier against penetration of compounds of molecular mass less than 50 kDa (with the exception of mycobacteria) (Franklin and Snow 2005).

Alkylation effect

Alkylation was revealed to vary antimicrobial efficacy in a different manner, depending on the length of alkyl tail and macromolecular system. For instance, alkylation of poly (alkyldimethyl(vinylbenzyl)ammonium chloride) with the longest alkyl radical C_{12} was shown to significantly enhance its antimicrobial efficacy (Ikeda et al. 1984c). However, Panarin and co-workers have found that the bacteriostatic properties of water-soluble cationic quaternary copolymers based on vinylamine, aminoalkyl methacrylates, and *N*-vinyl pyrrolidone with pendent quaternary ammonium groups are virtually independent on the length of the alkyl substituents at the nitrogen, while the activity of the monomers increases by several orders of magnitude

[1] It should be emphasized that poly(4-vinylpyridine) (PVP), prepared by free radical polymerization, should have medium polydispersity (PDI), i.e., the ratio M_w/M_n, where M_n is the number-average molecular weight: 1.5< PDI<2. At the same time, branched PEI, which is obtained by cationic step polymerization, has large PDI for polymers with high MWs. According to the data of Sigma-Aldrich Chemical Co, the values are as follows: M_w 2 kDa (by LS), M_n 1.8 kDa (by GPC), PDI=1.11; M_w 25 kDa (by LS), M_n 10 kDa (by GPC), PDI=2.5; M_w 750 kDa (by LS), M_n 60 kDa (by GPC), PDI=12.5. Thus, antimicrobial effect, which is observable for PEI with $M_w \geq 25$ kDa, should be averaged over the effect of polymers with molecular weights $M_w - M_n$ within polydispersity range.

Table 2 The ability of microbicidal surfaces modified with synthetic quaternary cationic polymers to kill microorganisms on contact

Polymer structure	M_w/M_n (kDa); PDI [a]	Surface	Microorganism	Killing percentage; microorganism loading (or cells/mL in suspension)	Ref.
	M_w 160;[b]	Glass, polyethylene, polypropylene, nylon and poly(ethylene terephthalate)	Airborne *S. aureus*, *S. epidermis*, *P. aeruginosa*, *E. coli*	90% to >99% in 2 min; for glass: aerosol 10^6/mL *S. aureus*, *S. epidermis*, *P. aeruginosa*; 10^7/mL *E. coli*; for : other surfaces 5.3×10^4 *S. aureus*, 5.3×10^3/cm^2 *E. coli*	Tiller et al. 2001, 2002
		Polyethylene, polypropylene, nylon and poly(ethylene terephthalate)	Waterborne *S. aureus*, *E. coli*	94% to >99% in 2 h; 1.07×10^5, *S. aureus*; 2.14×10^5/cm^2, *E. coli*	Tiller et al. 2002
	750/60;[b] PDI 12.5	Cotton, wool, nylon, polyester	Airborne *S. aureus*, *S. epidermidis*, *P. aeruginosa*, *E. coli*, *S. cerevisiae*, *Candida albicans*	85% to 99%;[c] aerosol 10^5/mL	Lin et al. 2003
	2.0/1.8 [b]; PDI 1.11	Cotton	Airborne *S. aureus*	36 – 70%;[c] aerosol 10^5/mL	
	M_n 22 [d]; 1.62	Glass, paper	Waterborne *E. coli*, *Bacillus subtilis*	>99-100% in 1h; 3.2×10^3 – 4×10^7/cm^2	Lee et al. 2004
	M_n 1.5; [d] PDI 1.16			85% in 1h; 2.9×10^5/cm^2	
	M_n = 10 (21; 35); [d] PDI 1.18 (1.21; 1.22)	Polypropylene	Waterborne *E. coli*	100% in 1h; 2.9×10^5/cm^2	Huang et al. 2007

[a] See footnote 'a' in Table 1

[b] See footnote 1 in main text

[c] Time of contact is not indicated

[d] Method GPC

with the increment of alkyl length from C_1 to C_{16} (Panarin et al. 1985). Cooper and co-workers have disclosed a parabolic relationship between biocidal activity of the synthesized quaternized poly(propyleneimine) dendrimers and the hydrophobic chain length of the quaternary ammonium groups (the highest potency was achieved for C_{10}) (Chen et al. 2000). Klibanov and co-workers have revealed that for alkylated quaternized PVP polymers, the length of alkyl tails should not be excessive (the optimal length was C_6) (Fig. 4) to avoid a huge decrease in the biocidal efficacy of antimicrobial surfaces derivatized with the polymers. This was explained by the stronger hydrophobic interactions and aggregation of polymer molecules with the quite large alkyl spacers on the surface (Tiller et al. 2001;

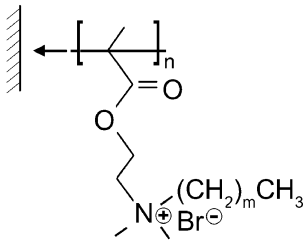


Fig. 5 Schematic representation of quaternized with alkyl bromide poly(2-(dimethylamino) ethyl methacrylate) (PDMAEMA) immobilized on a surface

Lewis and Klibanov 2005). Neoh and co-workers have explored influence of the different carbon chain lengths (from C_4 to C_{10}) at quaternized pyridine nitrogen on the activity of the synthesized microbeads (Hu et al. 2005). Recently, Tiller and co-workers have reported results on the effect of inherently non-biocidal satellite end alkyl groups of different length on the antimicrobial activity of the synthesized poly (methyloxazoline)s (PMOX) with the antimicrobial *N*,*N*-dimethyldodecylammonium (DDA) group at the diametrically opposite ends (Waschinski et al. 2005; Waschinski and Tiller 2005; Waschinski et al. 2008a, b). Studies of the aggregation behavior in solutions of PMOX–DDA with methyl, decyl, and hexadecyl satellite groups and also interactions of these polymers with model liposomes have shown that aggregation of polymer molecules is unlikely to be the reason for the different antimicrobial activity of the polymers; it was proposed that the satellite non-bioactive hydrophobic groups truly control the antimicrobial activity of the polymers preventing the penetration of the antimicrobial DDA group through a model membrane in the case of the long hexadecyl satellite group (Waschinski et al. 2008b).

One should mention that with the alkyl chains growth, several characteristic features and parameters of polymer behavior change. On the one hand, there is a boost of adsorption/absorption ability and lipophilicity, but on the other hand, this leads to variation of hydrophilic–hydrophobic balance of a polymer that can vary killing ability against different microorganisms in a different manner. Also, this leads to an increase in macromolecular aggregation, alteration of conformation behavior of a macromolecule on a cell surface, and penetration ability through OM/CW. Concurrence of these different tendencies may be assumed to result in different optimal alkyl tail lengths for different macromolecular systems to achieve higher antimicrobial efficacy against a given type of microorganism in solutions or on surfaces.

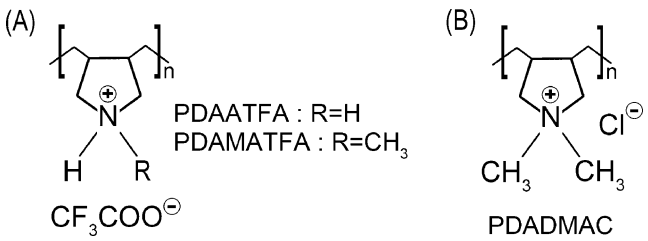


Fig. 6 **a** Poly(diallylammonium trifluoroacetate) (*PDAATFA*) and poly(diallylmethylammonium trifluoroacetate) (*PDAMATFA*). **b** Poly (diallyldimethylammonium chloride) (*PDADMAC*)

Hemolytic/toxic activity

In recent years, systematic studies have been initiated on some parameters modulating not only antimicrobial properties of synthetic quaternized polymers but also their hemolytic activity and toxicity to human cells. These investigations imply that biocompatibility is one of the necessary properties for a broad range of biomedical applications of the designed polymers. Sen et al. have methodically studied antimicrobial and hemolytic properties, and also toxicity to human cells of the synthesized amphiphilic quaternized pyridinium–methacrylate copolymers as a function of the spatial separation of the positive charge and the hydrophobic alkyl side chains in the copolymer (Sambhy et al. 2008). It has been found that locating the charge and tail on the spatially separated centers (i.e., side chain is on the acrylate group) results in a higher membrane-disrupting ability including antibacterial and hemolytic activities as well as in a substantial increase in mammalian cell toxicity. The highest selectivity (HC_{50}/MIC=34:1, HC_{50} is the hemolytic concentration lysing 50% of blood cells) was reported when the charge and the tail were on the same center (i.e., alkyl tail is on the pyridinium nitrogen) (Sambhy et al. 2008). Youngblood and co-workers have investigated the effect of hydrophilic properties of macromolecular systems on their antibacterial efficacy and biocompatibility; they have shown that copolymerization of vinylpyridine with strongly hydrophilic comonomers, hydroxyethyl methacrylate and poly (ethylene glycol) methyl ether methacrylate (followed by quaternization of PVP links), significantly improves both the efficacy and the biocompatibility (characterized by interaction with human red blood cells) of copolymers in comparison with the quaternized PVP, which is known to possess high antimicrobial efficacy but minimal biocompatibility (Sellenet et al. 2007; Allison et al. 2007).

Still there is no complete understanding of all the factors that are responsible for toxicity and control selectivity. For example, Klibanov and co-workers have shown (using two-color Live/Dead fluorescence assay) that bactericidal *N*-hexyl,methyl-PEI surface coating, although highly lethal to bacteria, has no appreciable detrimental effect on mammalian cells and related this to the much larger size of the latter (Milovic et al. 2005). Tew and co-authors believe that the antimicrobial polymer selectivity originates from different chemical content of the outer envelope of bacterial and eukaryotic cells, in particular very distinct phospholipid composition of bacterial and eukaryotic cells, that results in a more negative charge on the outer leaflet of bacterial cells than eukaryotic cells at the phospholipid level (Gabriel et al. 2007).

Amine structure

Since the 1980s, quaternization of amine groups of polymers has been considered as the main way for preparing polymers with the antimicrobial functions that allowed one to fix cationic charge on a polyelectrolyte and thus substantially enhance its activity (with the exception of guanidines and biguanide compounds). However, recently, macromolecular systems containing non-quaternary protonated amine groups in the links were reported, which exhibit quite high biocidal activity, unlike similar *N*-quaternized polymers. Gellman and co-workers have shown that polystyrenes containing protonated tertiary amine groups exhibited higher antibacterial activity than analogous *N*-quaternized polymers (Gelman et al. 2004). Kuroda and co-workers have synthesized amphiphilic random methacrylamide copolymers bearing protonated primary or tertiary amine groups, or quaternary ammonium groups and hydrophobic alkyl groups in the side chains. They have shown that the copolymers containing protonated primary or tertiary amine groups can be tuned to achieve high antimicrobial and minimal hemolytic activity (Fig. 7), unlike the copolymers of this series containing quaternary ammonium groups, which required a greater amount of hydrophobic comonomer to exhibit antimicrobial activity and had low selectivity (Palermo and Kuroda 2009). The results of a detailed study of the antimicrobial activity (against *E. coli* and *S. aureus*) dependence of the copolymers containing primary amine groups on varying mole fraction of alkyl side chains, as well as dye leakage from model vesicles dependence, were interpreted as evidence of the membrane-disrupting action of the methacrylamide random copolymers (Fig. 7) (Palermo et al. 2009). Importantly, antimicrobial activities and biocompatibility were shown to depend in a different manner on the mole fraction of the alkyl side chains and the length of alkyl groups (Table 1) (Palermo et al. 2009).

Timofeeva and colleagues have synthesized water-soluble cationic PDAAs containing pyrrolidine links with protonated secondary or tertiary amine groups (Fig. 6a) (Timofeeva 2002a, b, 2005) and revealed that these polymers are highly potent antimicrobials exhibiting high activity against a representative set of bacteria and fungi of *Candida albicans* species (optimal MIC_{100} values including data for both polymers range from 1.5 to 30 μg/ml), unlike the quaternary hydrophobic polymers of this series, in particular poly(diallyldimethylammonium chloride) (PDADMAC) (Fig. 6b) (Table 1) (Timofeeva et al. 2009). The secondary polyamine poly(diallylammonium trifluoroacetate) (PDAATFA) was shown to exhibit quite strong biocidal properties at different conditions, including aqueous solutions of moderate ionic strength (serum, 0.01 M/0.1 M) and aqueous-alkaline solutions (pH 10.5) (where the polymer

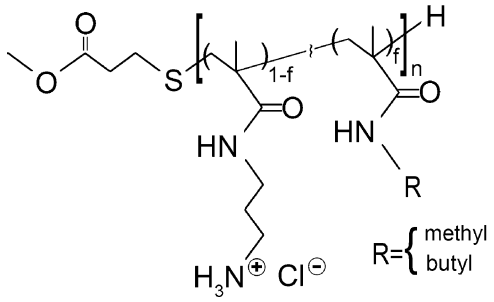


Fig. 7 Random copolymers of methacrylate monomers containing protonated primary amine group and alkyl group in side chains; *f* is mole fraction of alkyl side chains

presences as protonated polybase), unlike polymeric quaternary ammonium salts (PQAS). High biocidal efficacy of the non-quaternary PDAAs has been related to the essential role of hydrophilic NH_2^+/NH^+ groups in combination with the hydrophobic structure of pyrrolidine rings that results in strong intermolecular polycation–lipid, polycation–bilayer membrane electrostatic interactions enhanced by specific hydrogen bond interactions at different stages of action leading to more effective damage of structural organization and integrity of cell membranes (Timofeeva et al. 2009). The results of TEM study have supported the mechanism involving membrane-disrupting action of the PDAAs (Timofeeva et al. 2009). Characteristic TEM images of the bacterium *E. coli* treated with aqueous solutions of PDAATFA permit to reveal morphologic changes in the bacterial cell caused by the effect of bactericidal MIC_{100} and bacteriostatic (a half MIC_{100}) PDAATFA concentrations (Fig. 8). It is seen in Fig. 8 that the changes occurring in submicroscopic organization of the Gram-negative bacteria involve all structural components of the cell: OM, cytoplasmic membrane, cytoplasm, ribosomes, and nucleoid (Timofeeva et al. 2009).

The important role of hydrogen bonds in the polymer–bilayer membrane interactions was shown by Melik-Nubarov and co-workers in the dye-leakage study of interactions between anionic polyacids and phosphatidylcholine membranes in a slightly acidic media, where phosphate groups of phospholipids are protonated and, as a result, the lipid membrane becomes positively charged (Berkovich et al. 2009). It was shown that, in addition to electrostatic binding with the choline groups of lipid molecules and hydrophobic binding with the membrane, the polymer–membrane complex is stabilized by hydrogen bonds that results in the increase in permeability of the membrane (Berkovich et al. 2009).

Synthetic mimics of antimicrobial peptides

Lately, there has been an increasing interest in the synthetic mimics of the antimicrobial peptides (SMAPs) involving

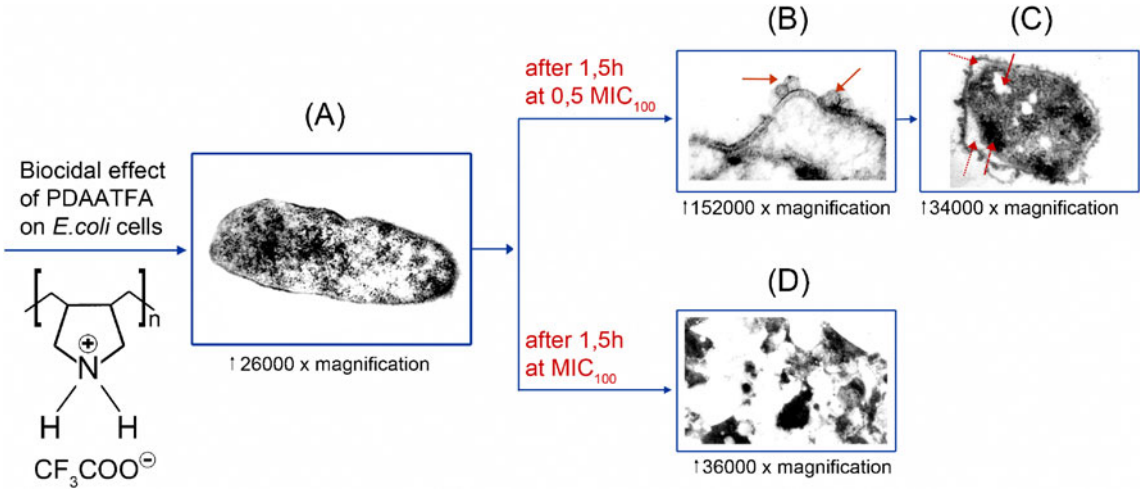


Fig. 8 Morphologic changes in the *E. coli* cells examined by transmission electron microscopy. *E. coli* culture was maintained during 1.5 h in solutions of PDAATFA (M_w=24 kDa) at the MIC_{100} bactericidal (125 μg/ml) or bacteriostatic (62 μg/ml) concentrations. When *E. coli* was maintained at the bacteriostatic concentration, it was made possible using the images to trace different morphologic changes in the cells. **a** Control culture. **b** Sticking of polymer to the outer membrane (noted by *arrows*). **c** Increase in periplasmic space (noted by *point arrows*) that is most probably evidence of a disturbance of the water–salt metabolism, increase in the image density of the cytoplasm (noted by *dashed arrow*), and the lysis zones in the cytoplasm (noted by *arrow*). **d** Cellular detritus, images of which are characteristic of the culture maintained at the bactericidal concentration MIC_{100}

synthesis of novel polymer mimics and investigation of mechanism of their antimicrobial action (Gabriel et al. 2007; Tew et al. 2010). It has been revealed by DeGrado and co-workers and Tew and co-workers that many antimicrobial HDPs and some of their mimics act in way of general disruption of the cell membrane (Gabriel et al. 2007; Tew et al. 2010). One strategy in developing polymer SMAPs with selective properties is to design polymers and oligomers that have highly amphiphilic conformation [named facially amphiphilic (FA)] in which the cationic hydrophilic and hydrophobic side chains segregate onto distinctly opposing regions or faces that appears to be important for insertion into and disruption of the cytoplasmic membrane (Tew et al. 2002). In the SMAPs series, antimicrobial polynorbornenes composed of FA monomers with protonated primary amine groups were synthesized (Fig. 9), and mechanisms of their action were investigated by Tew and co-workers (Tew et al. 2010). It was shown that fairly hydrophobic polymer **B** (Fig. 9b) is active and non-selective since it disrupts both mammalian and bacterial cell membranes (that was confirmed by measuring polymer-induced dye leakage from large unilamellar vesicles), while hydrophilic polymer **A** (Fig. 9a) is inactive (Ilker et al. 2004). The increase in the charge density on the monomer units by doubling or tripling protonated amine groups resulted in the polymer **A** becoming active against Gram-positive *S. aureus*, while the boost of hydrophilicity led to a significant decrease in hemolytic activity of the hydrophobic structure **B** (Al-Badri et al. 2008). Using dye-leakage, DLS, ITC, and fluorescence microscopy studies on vesicles and bacterial cells, it was concluded that interaction of fairly hydrophobic polynorbornenes, for example **B** (Fig. 9b), with synthetic vesicles or bacterial cells leads to their extensive aggregation, but complete membrane disruption does not appear to take place like upon other surfactants (Gabriel et al. 2008a). By modifying the structure of non-active polymer **A** (Fig. 9a), guanidinium analogues **C** were prepared (Fig. 9c) that remained potently antimicrobial, but were not strongly membrane disruptive and had low hemolytic activity (Table 1) (Gabriel et al. 2008b). A whole set of highly potent antimicrobial polynorbornenes was also reported with tunable activity and selectivity (Lienkamp et al. 2008). Two approaches to designing of the antimicrobial copolymers with higher hydrophobicity based on polyamine oxanorbornene were compared in which random copolymers were prepared by either copolymerization of two FA monomers or copolymerization of polar (with protonated primary amine group) and hydrophobic monomers (Gabriel et al. 2009). It was concluded that the balance of hydrophobic/hydrophilic areas at the local monomer is much more critical to attain high activity and selectivity of the polymer (Gabriel et al. 2009). The effect of different counterions and also the effect of molecular charge density (by adding more cationic groups to the monomer molecule) on the polynorbornenes activity/selectivity were studied (Lienkamp et al. 2009).

Antimicrobial surfaces

During the last decade, permanent non-leaching antimicrobial surfaces have been intensively developed. The trends on surface modifications have been highlighted recently in

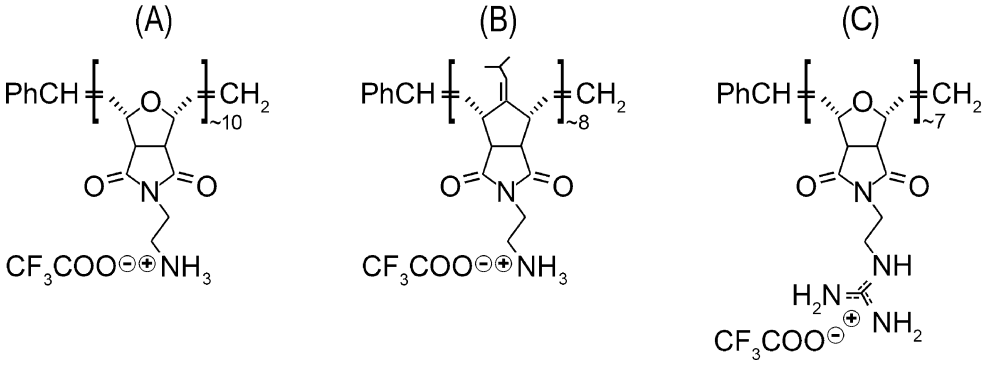


Fig. 9 Polynorbornenes. **a** Hydrophilic polymer **A**. **b** Hydrophobic polymer **B**. **c** Guanidinium modified polymer **C**

a review by Ferreira and Zumbuehl (2009). Here, we consider non-leaching microbicidal surfaces modified with synthetic antimicrobial polymers that are able to kill airborne as well as waterborne microbes on contact, and thus significantly limit bacteria colonization without release of antimicrobials into the environment. Several approaches have been designed for preparing non-leaching microbicidal material, such as covalent immobilization of antimicrobial polymers on a surface, depositing of polymers ("painting") onto a surface, and modification of a surface via graft polymerization of a monomer directly on the target surface. Since behavior of polymers attached to a surface differs from that in solution (which was discussed above), the factors of activity of the non-leaching antimicrobial polymeric coatings as well as some approaches to surface modification should be considered separately.

Surface modifications and factors of activity

A strategy for creation of non-release antimicrobial synthetic polymeric coatings has been developed in the Klibanov laboratory and included several necessary factors to make a surface modified with an antimicrobial polymer effective (Klibanov 2007). Unlike dilute solutions of hydrophilic–hydrophobic polymers, where there are intermacromolecular and polymer - solution molecules interactions, a problem arises when polymers are immobilized on the surface where hydrophobic attraction of macrochains can result in polymer aggregations, which are not able to interact with microbes. Another issue is that, due to the fixation of an end group of polymer molecule to a surface, polymer mobility is restricted, and therefore a minimal macrochain size is required to exert the full bactericidal effect via membrane-disrupting mechanism (unlike solutions where, as discussed above, some oligomers can also exhibit antimicrobial activity). In a series of studies devoted to making non-release permanently biocidal coatings by either covalent attaching or depositing ("painting") some common polymers onto surfaces of diverse materials, Klibanov and co-workers have shown that an immobilized polymer must be positively charged to prevent macromolecule self-associations, due to electrostatic repulsion, and to obtain more rigid ("protruding from the surface") conformation; also, macrochains must be hydrophobic, but not excessively, and sufficiently long (Klibanov 2007).

Tiller et al. have prepared bactericidal materials covalently coated with positively charged, due to alkylation, *N*-alkyl-PVP bromides using either graft copolymerization of 4-vinylpyridine with acryloyl moieties previously glass-bonded, followed by *N*-alkylation of grafted PVP, or covalent attachment of partially *N*-alkylated PVP to a surface (Fig. 4a) (Tiller et al. 2001). It has been shown that an alkyl radical must be moderately long (propyl through hexyl), while with longer alkyl chains (decyl through hexadecyl), polymer coating remained cloudy after alkylation and was inactive, which agreed with the view that positive macrochains must be moderately hydrophobic to avoid their self-associations. It has been also found that *N*-hexyl-PVP coating with shorter macrochains exhibited less activity (killed only 62±8% of the deposited *S. aureus* cells), while glass slides modified with *N*-hexyl-PVP of M_w 160 kDa, via each of the above methods, exhibited very high activity against representative set of bacteria (Table 2) (see 'Molecular weight effect' section) (Tiller et al. 2001). The range of bactericidal materials have included commercial polymers such as high-density polyethylene and low-density polyethylene, polypropylene, nylon, and poly(ethylene terephthalate) derivatized with hexyl-PVP (Table 2) (Tiller et al. 2002). Lin et al. have extended the findings to another common class, hydrophilic polymer branched PEI (Lin et al. 2002, 2003). Glass slide and textiles (cotton, wool, nylon, and polyester) treated with alkylated quaternized PEI (*N*-hexyl, *N*-methyl-PEI) (Fig. 4b) of sufficiently high $M_w \geq 25$ kDa exhibited biocidal efficacy toward airborne and waterborne bacteria and also fungi (Table 2) (here and below for PEI, see footnote 1) (Lin et al. 2002, 2003).

Park et al. and Haldar et al. have used non-covalent coating for the preparation of microbicidal materials by means of painting a surface with *N*-dodecyl,*N*-methyl-PEI dissolved in organic solvent, and the material biocidal action against *S. aureus* and *E. coli* has been shown not to be due to leaching of the polycations from the surface (Park

et al. 2006; Haldar et al. 2006). Slides painted with *N*-dodecyl,*N*–methyl-PEI of sufficiently high MW (M_w 750 kDa or 25 kDa) have been shown to be 100% effective against bacteria and influenza virus (belonging to an enveloped class, representatives of which are protected by a lipid membrane); significantly, the observed virucidal effect was found to be on contact without leaching of the polymer (Haldar et al. 2006).

Another common polymer, PDADMAC (Fig. 6b), possessing high absorbing/adhesion ability to negatively charged particles/surfaces was used for preparing antimicrobial coatings (Thome et al. 2003; van der Mei et al. 2008). Thome et al. have designed two approaches to antimicrobial treating of a polymer surface either by grafting PDADMAC to the surface via radical polymerization of the monomer on the surface or by coupling PDADMAC to the polymer surface; the resulting PDADMAC coatings reduce the settlement of bacteria, such as Gram-positive *Micrococcus luteus* and Gram-negative *E. coli* by a factor of 10^5–10^6 (Thome et al. 2003). Van der Mei et al. have prepared PDADMAC coatings by means of depositing the polymer onto glass slides via immersion in an aqueous PDADMAC solution of different concentration; the resultant PDADMAC coatings strongly enhance adhesion of the tested waterborne pathogens *Raoultella terrigena* and *E. coli* to coated glass slides applied from 1, 100, or 500 ppm aqueous solutions and pathogen *Brevundimonas diminuta* to a positively charged slide with PDADMAC coating applied from a 500 ppm solution, as well as reduce the bacterial viability by the positively charged quaternary ammonium (QA) groups in the coating (van der Mei et al. 2008).

Neoh et al. have developed a simple method for preparing polymeric microbeads with a spherical shape and narrow particle distribution based on poly(4-vinyl pyridine)/poly(vinylidene fluoride) (Hu et al. 2005). After quaternization of pyridine groups on the surface of the microbeads with alkyl bromides of different carbon chain lengths (from C_4 to C_{10}), the microbeads possessed effective antibacterial (against *E. coli*) and antifungal (against *Aspergillus niger*) properties, which were sustained in repeated applications (Hu et al. 2005).

The Matyjaszewski laboratory and Russell laboratory have jointly developed a strategy for producing non-leaching antimicrobial materials, which uses atom transfer radical polymerization (ATRP) method to perform living radical polymerization and obtain polymer chains of controlled molecular weights and low polydispersities, either from a surface ("grafting from" technique) or on a surface ("grafting onto" technique) (Lee et al. 2003, 2004; Huang et al. 2007, 2008; Murata et al. 2007). In these studies, surface charge density, i.e., concentration of QA groups obtained due to quaternization of polymers, has been shown as a critical factor in the antimicrobial efficacy of the surfaces. Characteristic feature of the polymer distribution on a surface has been also found to affect the killing efficiency of the modified surface.

Lee et al. have prepared antimicrobial surfaces, glass and paper, coated by non-leachable poly(2-(dimethylamino) ethyl methacrylate) (PDMAEMA) via ATRP "grafting from" technique, which included immobilization of an ATRP initiator to a surface, followed by polymerization of 2-(dimethylamino)ethyl methacrylate (DMAEMA) directly on the surfaces and quaternization of tertiary amino groups of the prepared PDMAEMA with an alkyl halide to produce a large concentration of QA groups on the polymer-modified surfaces (Fig. 5) (Lee et al. 2004). A modified piece of paper and glass slide exhibited high activity against *E. coli* or *B. subtilis* (Table 2); the permanence of the antimicrobial activity was shown through repeated use of a modified glass without significant loss of activity (Lee et al. 2004). Huang et al. have prepared antimicrobial plastics (polypropylene) coated with non-leachable quaternized PDMAEMA via "grafting from" method (Huang et al. 2007). It has been shown that, with the same grafting density, the biocidal activity against *E. coli* of the resultant surfaces is controlled by the number of QA units, which was dependent on the MW (i.e., DP) of grafted polymers, and the minimal macrochain size is needed at the surface to attain killing efficacy (Table 2) (Huang et al. 2007). Murata et al. have also used "grafting from" technique to prepare polymer brushes on inorganic surfaces via surface-initiated ATRP of DMAEMA, followed by quaternization of tertiary amino groups of the PDMAEMA with alkyl bromides (Murata et al. 2007). The macrochain length and density, as well as surface charge density, have been assessed to ascertain the mechanism of bactericidal action against *E. coli* (Murata et al. 2007). Huang et al. have prepared antimicrobial glass surfaces treated with PDMAEMA/poly (3-(trimethoxysilyl)propyl methacrylate) (TMSPMA) copolymers using "grafting onto" technique (Huang et al. 2008). PDMAEMA/TMSPMA block and random copolymers were prepared by ATRP, followed by covalent attachment to a glass surface through reaction of the trimethoxysilyl groups with surface silanol groups and quaternization of PMAEMA amine groups (in an alternative way, block copolymers with quaternized PDMAEMA were attached to surfaces) (Huang et al. 2008). Importantly, for a similar density of QA groups (which was kept constant), the biocidal activity of the grafted surfaces against *E. coli* has been shown not to depend on the varying length of PDMAEMA macrochains as well as the chemical structure of copolymer. The surfaces prepared by the "grafting onto" method were shown to possess higher biocidal efficacy than surfaces prepared by "grafting from" at comparable QA densities; it was assumed to be related to uneven distribution of QA groups with local centers of high

QA group concentrations in the case of "grafting onto" surfaces (Huang et al. 2008).

Mechanism of action

Klibanov and co-workers have employed two-color Live/Dead fluorescence test to verify the mechanism of action against *S. aureus* and *E. coli*, and revealed that surfaces covalently derivatized with *N*-hexyl,*N*-methyl-PEI (PEI with M_w 750 kDa) or painted with *N*-dodecyl,*N*-methyl-PEI (PEI with M_w 750 kDa) killed bacteria by rupturing their cellular membrane (Milovic et al. 2005; Park et al. 2006; Klibanov 2007). By monitoring loss of bacterial cells constituent, Neoh et al. have concluded that the microbeads based on PVP kill *E. coli* cells by membrane disruption (Hu et al. 2005). Atomic force microscopic images of *E. coli* cells on glass surfaces, modified with "grafted from" PDMAEMA, also support the hypothesis of membrane-disrupting action of quaternary amines (Lee et al. 2004). At the same time, taking into consideration the length of PDMAEMA macrochains (obtained by "grafting from") of ~10 nm, which is smaller than the thickness of the *E. coli* cell envelope (~46 nm, from CM to outside of OM) (Matias and Beveridge 2005; Matias et al. 2003), Murata et al. concluded that QA surface-active groups destabilize the membrane of the *E. coli* cell by exchange with the divalent cations, causing cell death (Murata et al. 2007). Although Huang et al. have shown using the Live/Dead method that the surface-deposited QA groups, obtained by PDMAEMA "grafting onto", kill bacteria by rupturing their membranes, nevertheless they noted that the biocidal action of short QA chains (DP=97, i.e., ~24 nm) could not be due to penetrating the *E. coli* cell membrane, thus supporting the ion-exchange mechanism rather than penetration (Huang et al. 2008). Summarizing, although damage of bacterial cells were identified using different methods as a final result of quaternary amine brush action, there is still no clear understanding of their antimicrobial mechanism.

Antimicrobial activity—type of microorganism

It was shown in the overwhelming number of investigations that biocidal activity of antimicrobial cationic polymers varies significantly between different types of microorganisms. Susceptibility to biocides is first of all considered in terms of the structure and chemical nature of cell outer envelope altering greatly for different types of microbes (McDonnel and Russell 1999; Maillard 2002; Franklin and Snow 2005). McDonnell and Russell note that, as a whole, Gram-negative microorganisms exhibit stronger resistance to antiseptics and disinfectants than Gram-positive ones (with the exception of Gram-positive mycobacteria), and fungi of the *Candida* genus occupy an intermediate position (McDonnel and Russell 1999). Franklin and Snow stress that the high resistance of Gram-negative bacteria is due, to a significant extent, to the structure of the outer membrane whose porin channels slow down molecular diffusion and, as a result, limit the penetration of antibacterial substances into the cell (Franklin and Snow 2005).

However, recently, some data on the antimicrobial activity of polymers have been reported, which indicate that the aforesaid order of the polymer activity behavior can be altered depending on the chemical structure and MW of polymers. For instance, several appropriately hydrophobic polynorbornenes, oligomers (M_n 3 kDa), and polymers (M_n 10 kDa) were shown to be more potent against Gram-negative *E. coli* than Gram-positive *S. aureus* (or inactive against the latter at all), and the distinctions were much stronger for polymers with M_n 10 kDa (Lienkamp et al. 2008). Hydrophilic polymeric base branched PEI, found to be inactive on a surface against *S. aureus* (Lin et al. 2002), has been shown (PEI with M_w 45 kDa) to exhibit noticeable fungicidal effect in solution while being inactive there against *E. coli* and *S. aureus* (Timofeeva et al. 2009).

Biocide usage—antibiotic resistance

For a long time, it has been broadly accepted that biocidal polymers (like small molecular weight quaternary ammonium compounds, QACs) with membrane disruption activity may be characterized by the non-specific mechanism of their brute-force action, unlike other antimicrobials, antibiotics in particular, targeted at specific metabolic processes in bacteria (Hugo 1999; McDonnel and Russell 1999). The usage of the latter has been assumed to be responsible for drug resistance in bacteria (McDonnel and Russell 1999; Russel 2001, 2002, 2003). However, during the last decade, the issue has been debated concerning possible relationship between biocide usage and antibiotic resistances. The suggestion has arisen that introduction of biocides into clinical and other practice has been also responsible for the selection of antibiotic-resistant bacteria (Russell 2002, 2003; Gilbert and McBain 2003; Gilbert and Moore 2005). In this relation, Gilbert and Moor emphasize the important difference between the mode of antimicrobial action of biguanides (in particular, PHMB) and other cationic quaternized membrane active biocides, QACs (Gilbert and McBain 2003; Gilbert and Moore 2005). It was stressed that PHMB interacts only superficially with the lipid bilayer altering fluidity through cation displacement and head-group bridging leading to a generalized and progressive leakage of cytoplasmic materials to the environment, whereas the mode of action of QACs against bacterial cells involves a general perturbation of lipid bilayer membranes constituting the bacterial cytoplasmic membrane and the OM of Gram-negative bacteria, and

therefore thought to be susceptible to resistance mechanisms mediated through multidrug efflux pumps (Gilbert and McBain 2003; Gilbert and Moore 2005). At the same time, Russell supposes that further research is needed to explore any possible linkage between biocide usage and drug resistance (Russell 2002, 2003).

Application

Cationic quaternized amphiphilic polymers/copolymers containing ammonium/phosphonium functional groups with rather long alkyl substituents, or hydrophobic alkyl side tails, as well as polymers with antimicrobial groups, such as *N*-halamine, phenol derivatives, have found wide application in different fields.

Of these, one of the most important is infection control in clinics, hospitals, food industry, and domestic settings. Water-soluble cationic ammonium polymers, polymeric guanidines and biguanides, and PQAS have found use as disinfectants (along with the ordinary low molecular weight QAS and antimicrobials of another class) (McDonnel and Russell 1999; Tashiro 2001; Block 2001; Gilbert and Moore 2005). The disinfecting agents should possess such properties as noticeable efficacy as well as broad spectrum of activity, low toxicity, and environmental safety. Unfortunately, none of the currently known disinfecting compounds of any chemical class meets all the expectations. In comparison to the ordinary low molecular weight biocidal QAS, PHMB and PQAS are characterized by greater efficacy, in particular, rather high efficacy against Gram-positive bacteria, and low toxicity, especially PHMB. PHMB and some of PQAS are known to be non-irritative for skin and are described as non-mutagenic and non-carcinogenic (McDonnel and Russell 1999; Merianos 2001; Gilbert and Moore 2005). But a quite narrow spectrum of antimicrobial activity, including limited efficacy against Gram-negative bacteria and yeast, and the absence of tuberculocidal and sporicidal activity, as well as a sharp decline of efficacy in presence of organic materials, in particular, blood, and incompatibility with soap because of alkalinity seriously limit their use (Block 2001; Merianos 2001; Lauzardo and Rubin 2001). Disinfecting agents based on PQAS are used for disinfection of non-critical surfaces such as floors, walls, and equipment surfaces in hospitals, nursing homes, public places, as a rule, in combination with compatible detergents to permit one-step disinfecting and cleaning operation (Merianos 2001; Block 2001). Excellent toxic profile of the polymeric biguanides and guanidines, their non-irritation for skin, and oral tolerance allow one to widely deploy them, especially PHMB, in clinical applications such as treatment of *Acanthamoeba keratinitis* (Larkin, et al. 1992) as well as beer glass sanitizer, general disinfecting agents in the food industry, and for the disinfection of swimming pools (Block 2001; Gilbert and McBain 2003). In Russia, disinfecting compositions based on the polymeric guanidines have found wide uses in hospitals, food industry, agriculture, and for wood protection, restoration of buildings, and others (Vointseva and Gembitsky 2009).

Polymers with *N*-halamine functional groups, including such heterocyclic *N*-halamine derivatives as oxazolidinones, imidazolidinones, hydantoins, and spirocyclic amines, demonstrated long-term stability and broad spectra of antimicrobial activities, including cysticidal and sporicidal ones dependent on the compound structure, which can be regenerated by exposure to an aqueous-free chlorine or bromine solution. Compared with the free halogens, organic *N*-halamines are more stable, less corrosive, and have much less tendency to generate halogenated hydrocarbons (Worley and Sun 1996; Sun et al. 2001; Sun and Sun 2001a, c, 2002a; Chen and Sun 2006; Kenawy et al. 2007). The potential uses of *N*-halamine polymers are extensive and include medical, dental, and industrial surfaces as well as textiles, paper, and water filter disinfection (Worley and Sun 1996; Kenawy et al. 2007).

A great number of the works over two decades until 2007 concerning applications of antimicrobial polymers have been described in detail by Kenawy et al. (2007). The major applications are as follows: use of developed water-insoluble polymeric materials with antimicrobial groups such as *N*-halamine, phenol derivatives, quaternary ammonium, or phosphonium groups for water and wastewater treatment that allows one to substitute current chlorine or water-soluble disinfectants and thus to prevent the residual toxicity of the agents (Worley and Sun 1996; Nonaka et al. 1997; Grapski and Cooper 2001; Kenawy et al. 2002; Sun and Sun 2002a, b; Kenawy and Mahmoud 2003; Chen et al. 2003, 2004); uses as various macromolecular carriers for drugs delivery with known antibiotics either embedded or covalently attached to the backbone, or as terminal groups (Woo et al. 2002; Dizman et al. 2005; Kenawy et al. 2007), as well as designing of new polymer drugs, in particular, based on polyelectrolyte complexes (Panarin and Kopeikin 2002); uses for medical device coatings that release antimicrobial agents (i.e., active release mechanism) such as antibiotics, silver ions, antibodies, and nitric oxide for reducing the incidence of implant-associated infection (Hetrick and Schoenfisch 2006); uses for antimicrobial food packaging (Kenawy et al. 2007; Bordenave et al. 2010); uses for treatment of textiles and fibrous materials to provide them with antimicrobial properties, for instance, materials and surfaces modified by polymers with *N*-halamine functional groups, either by means of copolymerization of polymerizable *N*-halamines with fabrics, cellulose, and glass or by treating materials (paper, cotton,

silica gel, cellulose), can effectively inactivate pathogenic microorganisms such as bacteria, fungi, and yeasts as well as virus particles (Fig. 10) (Sun et al. 2001; Sun and Sun 2001a, b, c, 2002b; Worley et al. 2005; Chen and Sun 2006; Liang et al. 2006, 2007; Kenawy et al. 2007; Kocer et al. 2008; Ren et al. 2008; Kou et al. 2009).

Along with the antimicrobial coatings based on active release of such antimicrobials as silver ions and antibiotics, or polymers with *N*-halamine functional groups, effective non-leaching antimicrobial polymeric coatings, which have been developed during the last decade, might find a wide application. The potential uses of the non-leaching immobilized polymeric coatings include biomedical supplies, environmental protection (building interiors, ventilation ducts, etc.), water treatment, household items and children's toys, consumer products, and many others. Of these, antimicrobial coatings for medical device are the ones of most importance because of high risk of infections related especially to urinary catheters, cardiovascular devices, and hip replacement devices (Ferreira and Zumbuehl 2009). However, studies with non-release cationic polymeric coatings were performed for few bacterial species, and further investigations are needed to determine their total efficacy as well as their biocompatibility to allow us to use them for medical device applications (Ferreira and Zumbuehl 2009).

Conclusion and future developments

In the last decade, many works have appeared concerning the synthesis of new antimicrobial macromolecular systems and investigations of their biological activity. This resulted in an active use of well-studied biocidal polymers and copolymers, water-soluble and insoluble, containing quaternary ammonium/phosphonium salts, other antimicrobial functional groups, and *N*-halamine groups in different areas. Various approaches were developed to create non-leaching antimicrobial coatings based on common modified or synthesized quaternized polymers. The prepared non-release polymeric coatings have been shown to be promising, and one may believe that this research field will be intensively developed.

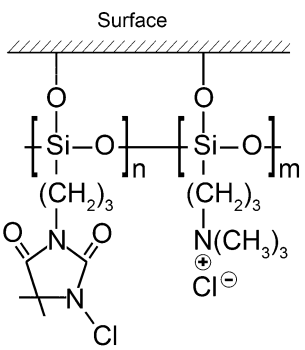


Fig. 10 Schematic representation of copolymer of an *N*-halamine siloxane with trimethylammonium salt siloxane coated on a surface

At the same time, there is still a great need for creation of new non-toxic macromolecular disinfectants of different levels with broad spectra of antimicrobial activity, including Mycobacterium tuberculosis that would retain their efficacy under different conditions (including ionic and alkali solutions) and could prevent contagion and microbial contamination in hospitals and public settings.

Along with the trends in development of macromolecular systems either quaternized or functionalized with bioactive groups, in recent years, new moderately hydrophobic polymer structures have been synthesized containing links with the protonated primary or secondary/tertiary amine groups, which exhibited rather high antimicrobial activity. It is of interest that some of the quaternary analogues of these polymers do not exhibit or have very low antimicrobial activity. Thus, the antimicrobial activity of the new polymers appears to be attained not by the boost of their hydrophobic properties, as it was observed with the quaternary active polymers, but due to the presence of hydrophilic protonated amine groups in combination with the suitable structure of the moderately hydrophobic links. Importantly, the shift of the hydrophobic–hydrophilic balance in the line of hydrophilicity significantly reduces toxicity of the non-quaternary polymers while increases their selectivity. One may suppose that these properties make non-quaternary bioactive polymers quite promising for biomedical applications in the areas where biocompatibility is one of the decisive factors. Therefore, further intensive studies of such macromolecules are of great interest.

A rather large class of polymers was referred to as membrane active agents. During the last decade, many data were received (often using experiments on polymer-induced dye leakage from model liposomes), which support the membrane-disrupting mechanism as a final result of antimicrobial action of the quaternary biocidal polymers as well as some non-quaternary polymers with protonated amine groups (although some new contradictory data have been received recently), whereas the proper mechanism of the interactions between membrane active macromolecules and bacterial or mammalian cells, or model liposomes, is not yet clear to the end. It may be expected that, due to progress in modern biophysical techniques, the studies of antimicrobial activity of polymers, either in solution or immobilized on surface, will provide interesting data and possibly new insight into the mechanisms of their antimicrobial action.

Research activity will be focused also on the studies of cytotoxicity to human cells of synthetic antimicrobial polymers to attain high selectivity and biocompatibility. These factors as well as more clear understanding of the mechanism(s) of antimicrobial action of polymers are necessary to design and synthesize innovative effective antimicrobial systems, which are environmentally safe and have no negative impact on human health.

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