Chapter 5
Polymeric Antimicrobials with Quaternary Ammonium Moieties

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Abstract Pathogenic microorganisms develop over time a resistance especially to the antimicrobial substances used abusively. Medical education and preventive medicine practiced by doctors is complemented by the research activity of chemists, pharmacists and physicists regarding the discovery of new antimicrobial substances. In order to minimize environmental toxicity and short duration of action characteristic of antimicrobial agents with low molecular weights, specialists focused over last few years on conceiving and studying of polymeric materials with antimicrobial properties.

This updated review refers to certain quaternary ammonium compounds synthesized based on natural or synthetic polymers, especially to those biocompatible. Polymers like cellulose, chitosan, dextran, pullulan, starch, cashew gum, poly(lactide), poly(amidoamine), poly(urethane), poly(siloxane), poly(methacrylate) or poly(ethylene) were modified with cationic quaternary ammonium moieties by different methods. Due to physicochemical properties of polymers or microenvironment factors, these quaternary ammonium containing polymers specifically interfere with the metabolism of a wide range of bacteria and fungi. Often, in order to increase the efficiency of these antimicrobial polymeric materials, the synergistic action of the quaternary ammonium groups with other groups or chemical compounds is considered.

Keywords Antimicrobials · Polymers · Quaternary ammonium · Bacteria · Fungi

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5.1 Introduction

Control and prevention of microbial contamination represent major challenges for researchers, doctors, and population. Over time, pathogenic microorganisms have developed some features that have helped them to survive even to the most aggressive antimicrobials of time. Usually, after a long period of efficient usage of new antimicrobials, resistance to those substances develops. Antimicrobial agents with low molecular weight generally have a series of disadvantages like environmental toxicity and short term antimicrobial capacity (Kenawy et al. 2007). In order to minimize these problems, specialists have focused in last years on design and study of polymeric materials with enhanced antimicrobial efficacy, reduced toxicity toward organisms or environment, and prolonged action time, compared with their small molecular weight counterparts (Jain et al. 2014). In this respect, various methods of introducing atoms or functional groups on polymer chain in order to obtain materials able to destroy structural integrity of the bacteria, fungi or algae have been tested. Antimicrobial macromolecular substances have been classified as: quaternary ammonium polymers, guanidine polymers, polymers designed to mimic natural peptides, halogenated macromolecules, polymers containing phosphonic or sulfonic derivatives and silver ion bearing polymers (Munoz-Bonilla and Fernandes-Garcia 2012; Siedenbiedel and Tiller 2012).

Attachment of substances with potential antimicrobial activity to the surface of microorganisms is possible due to the specific interactions between extracellular polymeric components like exopolysaccharides, proteins, lipids or humic substances, and reactive functional groups of antimicrobials. After cell surface adhesion, antimicrobial agents can act by various mechanisms: breakdown of cell membrane structure, inhibition of protein synthesis or metabolic pathways, interference with synthesis of nucleic acids, etc. Due to the surface negative charge of microbial entities, the most probable and efficient interactions will be with cationic contact surfaces. This category also includes quaternary ammonium compounds. Cationic quaternary ammonium antimicrobial agents are part of some formulations used as disinfectants, which act through electrostatic attraction, infiltration and diffusion (Rajkowska et al. 2016).

Importance of this type of materials is evidenced by a large number of reviews that generally discussed various aspects of mechanism of action and medical applications of antimicrobial polymers, as well as of quaternary ammonium based materials (Kenawy et al. 2007; Timofeeva and Kleshcheva 2011; Kenawy et al. 2014; Xue et al. 2015; Santos et al. 2016; Francolini et al. 2017; Jiao et al. 2017).

Present chapter covers most recent studies, predominantly over past 5 years, focused on natural or synthetic quaternary ammonium polymeric compounds and factors that influence their antimicrobial activity. It is generally assumed that molecular weight variation of polymers, hydrophilic-hydrophobic balance, conformation of polymer chain in solution, strength and type of intramolecular and intermolecular interactions will influence antimicrobial efficacy of polymers containing quaternary

ammonium moieties. Also, this chapter highlighted the diversity of polymers, especially those biocompatible that can be modified with quaternary ammonium groups to create new antimicrobial materials.

5.2 Polymeric Antimicrobials with Quaternary Ammonium Moieties

Specialized literature reports that various modifications of polymers in solution or in bulk, enhance their antimicrobial activity. From this point of view, antimicrobial applications of polymers with quaternary ammonium moieties are represented by coatings, modified surfaces and solutions. If, in some cases, coating implies application of electrospinning, dip-coating and polyelectrolyte complex formation onto polymeric support; in other ones, modified surfaces resulted from covalent linkage of quaternary ammonium salts directly onto polymeric support. Polymers with quaternary ammonium moieties and potential antimicrobial properties were synthesized from solution, purified by dialysis, concentrated and precipitated in non-solvent, filtered, dried for solvent removal, stored and then dispersed or dissolved in suitable solvent for antimicrobial tests.

In order to prevent biofouling phenomenon, researchers use different strategies to improve antibacterial properties of surfaces *via* creation of superhydrophobic surfaces; incorporation and controlled diffusion over time of biocide compounds from surfaces; and conjugation of antibiotic functional groups on surfaces. By such modifications, bacterial adhesion is suppressed, biocide leaching is initiated, and contact killing is activated (Lichter et al. 2009). Cationic quaternary ammonium groups could be immobilized or anchored onto polymeric chains *via* different approaches: chemical coupling reactions, surface initiated polymerization of monomers bearing antibacterial moieties and post-polymerization modifications to synthesize surface-tethered antimicrobial polymer brush (Gettings and White 1987; Lee et al. 2004; Lenoir et al. 2006; Wiarachai et al. 2012; Yao et al. 2010). In next sections, polymeric antimicrobials with quaternary ammonium moieties are classified and analyzed according to the nature of polymer matrix: natural or synthetic.

5.3 Antimicrobials Based on Natural Polymers

Natural polymers like cellulose, chitosan, dextran, starch, pullulan, gum, poly(lactic acid) are suitable for chemical modifications with quaternary ammonium moieties. The new polymers are characterized by improved biological, chemical and physical properties compared with their precursors *viz* better water solubility, moisture absorption, flocculation, nontoxicity, biocompatibility and antimicrobial activity.

5.3.1 Cellulose Type Antimicrobials

Cellulose is a polysaccharide with linear chain structure consisting of $\beta(1\rightarrow4)$ linked D-glucose units, and is a suitable macromolecule for chemical changes for applications in pharmaceutical industry in form of membrane, fibers or hydrogels. Meng et al. (2015) described a one-step modification method for direct covalent linking of quaternary ammonium salts onto membrane surface of regenerated cellulose, based on alkoxy silane polycondensation reaction and silane coupling agents like trimethoxysilylpropyl trimethyl ammonium chloride and trimethoxysilylpropyl octadecyldimethyl ammonium chloride. Reaction mechanism ensured chemical coupling of cationic antibacterial alkyl groups responsible for enhanced hydrophobicity of regenerated cellulose membrane and infiltration of ammonium groups through hydrophobic membranes of tested microorganisms. In same culture conditions, it was reported that *Staphylococcus aureus* ATCC 6538 more easily formed colonies on modified cellulose membrane compared with *Escherichia coli* DH5α. In addition, length of hydrophobic alkyl chains from regenerated cellulose membranes modified with trimethoxysilylpropyl trimethyl ammonium chloride or trimethoxysilylpropyl octadecyldimethyl ammonium chloride induced different behavior against the viability of bacteria. Thus, viability of *S. aureus* or *E. coli* was about 99% in presence of membrane modified with trimethoxysilylpropyl trimethyl ammonium chloride, while membrane modified with trimethoxysilylpropyl octadecyldimethyl ammonium chloride reduced the cell viabilities up to about 0.5%.

In order to avoid growth of pathogenic microorganisms on skin, researchers designed a hydrogel as a component of diapers, based on blending of native cellulose solution with quaternized cellulose solution (Peng et al. 2016). In this way, novel hydrogel formed by chemical cross-linking in aqueous solution of NaOH-urea mixture and in presence of epichlorohydrin, represented a potential antimicrobial material with improved mechanical properties, too. Varying molecular weight and substitution degree of quaternized cellulose (M_w = 94000 g/mol or 256000 g/mol; DS = 0.23–0.69), different hydrogels encoded as Gel9–2, Gel9–4, Gel9–6, Gel25–2, Gel25–4, and Gel25–6 were prepared and microbiologically tested. Quaternary ammonium groups introduced in hydrogel network by quaternization reaction of cellulose with 3-chloro-2-hydroxypropyl-trimethylammoniumchloride were responsible for inhibition of *Saccharomyces cerevisiae* cultures. The best antibacterial activity of Gel25–6 sample compared with other hydrogel samples suggested that a higher substitution degree of quaternized cellulose has intensified electrostatic type interactions between polycationic quaternary ammonium groups of cellulosic hydrogel and anionic components of microbial membranes leading to disruption and death of cells.

5.3.2 Chitosan Type Antimicrobials

As a natural material derived from chitin, chitosan is a non-toxic, biodegradable and linear macromolecule with cationic behaviour and comprises of β-(1 → 4) linked copolymer of 2-acetamido-2-deoxy-D-glucopyranose units and 2-amino-2-deoxy-D-glucopyranose, often used to design biocompatible materials. Even if it has antimicrobial properties, opportunity to enhance them was exploited by insertion of quaternary ammonium groups. Usually, chitosan is functionalized by quaternization, carboxymethylation, acylation or sulfhydrylation of amino groups in C_2 position of glucopyranose unit. Chitosan based materials with quaternary ammonium moieties are diverse in shape, state or functionality: hydrogels (Mohamed et al. 2015; Fan et al. 2015), fibers based on polyelectrolyte complexes (Ignatova et al. 2016), chitosan ammonium salts (Tang et al. 2015; Li et al. 2015; Tan et al. 2016; Li et al. 2016; Wang et al. 2016; Tang et al. 2016; Chen et al. 2016; Oyervides-Munoz et al. 2017) or copolymers (Song et al. 2016).

Hydrogels represent 3-D networks able to retain large amounts of biological fluids or water, being used in different fields such as biomedical devices, food packing or water purification. Also, quaternized chitosan based hydrogels serve as antimicrobial or wound healing materials. When Mohamed et al. (2015) designed hydrogels based on *N*-trimethyl ammmonium chitosan chloride and poly(vinyl alcohol) in weight ratios of 1:3, 1:1 and 3:1, they used glutaraldehyde in weight ratio of 1–5% as chemical cross-linking agent to ensure formation of chemical bonds between glutaraldehyde and hydroxyl groups of poly(vinyl alcohol) or residual amino groups from *N*-trimethyl ammmonium chitosan chloride. Beside different physico-mechanical properties, resulted materials exhibited specific antibacterial or antifungal activities. Thus, antimicrobial activity of *N*-trimethyl ammmonium chitosan chloride/poly(vinyl alcohol) hydrogels was higher than *N*-trimethyl ammmonium chitosan chloride itself, while antimicrobial effect increased with glutaraldehyde concentration.

Fan et al. (2015) performed gamma radiation-crosslinking to prepare hydrogels based on solution of hyaluronic acid and quaternary ammonium chitosan, poly(ethylene oxide) and poly(vinyl alcohol). Preliminary, quaternary ammonium chitosan was synthesized from chitosan and *N*-(3-chloro-2-hydroxypropyl)trimethyl ammonium chloride in NaOH solution. Then, solution of quaternary ammonium chitosan mixed with poly(vinyl alcohol)/poly(ethylene oxide) solution was subjected to irradiation with a ^{60}Co source. The sample irradiated with a dose of 40 kilograys was tested against *E. coli* RCMB 000107 and *S. aureus* RCMB 000106. In both cases, polymeric hydrogels containing quaternary amino $N^+(CH_3)_3$ groups showed a clear inhibition zone compared with poly(vinyl alcohol)/poly(ethylene oxide) hydrogel. Due to excellent moisture properties, these hydrogels were proposed as valuable materials for wound dressing applications.

By applying electrospinning, dip-coating and polyelectrolyte complex formation, Ignatova et al. (2016) designed various fibrous materials with antimicrobial and antioxidant properties, based on quaternized chitosan derivative named

N-trimethyl ammonium chitosan iodide, poly(3-hydroxybutyrate), caffeic acid and *k*-carrageenan. Thus, system represented by caffeic acid /poly(3-hydroxybutyrate) fibers was coated with *N*-trimethyl ammonium chitosan iodide/*k*-carrageenan polyelectrolyte complex, while system based on poly(3-hydroxybutyrate) fibers was coated with *N*-trimethyl ammonium chitosan iodide/*k*-carrageenan complex containing caffeic acid. Ability of fibrous materials containing *N*-trimethyl ammonium chitosan iodide and/or caffeic acid to inhibit the growth of *E. coli* and *S. aureus* was due to the fact that caffeic acid and *N*-trimethyl ammonium chitosan iodide exercised synergic antibacterial activity (bacteriostatic or bactericidal). Both materials containing caffeic acid and quaternized chitosan suppressed bacteria growth after 3–4 h of exposure. In addition, by studying *S. aureus* cells adhesion to the surface of fibrous materials, it was observed from scanning electron microscopy images that a large number of viable *S. aureus* cells with unaltered morphology adhered on surface of hydrophobic neat poly(3-hydroxybutyrate) fibers. In return, on contact with surface of treated poly(3-hydroxybutyrate) fibers, adhesion of pathogenic *S. aureus* bacteria was suppressed such that no bacteria or only a limited number of adhered bacteria were observed.

When antimicrobial properties of molecular iodine were combined with those of *N*-(2-hydroxy) propyl-3-trimethylammonium chitosan chloride, a new material with stronger antimicrobial activity was obtained (Tang et al. 2015). Thus, a stable charge transfer complex of *N*-(2-hydroxy) propyl-3-trimethylammonium chitosan chloride with iodine resulted by combination of those two components in a molar ratio of 1:1.33, due to the attraction between large electron cloud density of iodine and positive charge of quaternary ammonium salt. By testing antibacterial efficacies of samples against *S. aureus* and *E. coli*, it was observed that the antibacterial inhibitory effect increased in order: chitosan > *N*-(2-hydroxy) propyl-3-trimethylammonium chitosan chloride > *N*-(2-hydroxy) propyl-3-trimethylammonium chitosan chloride-iodide. Other approach to improve antimicrobial activity of chitosan consisted of synthesis of chitosan quaternary ammonium salts with halogens: chitosan halo-acetates and chitosan halo-1,2,3-triazoles (Tan et al. 2016; Li et al. 2016). In this way, electronegativity of halogenic substituent groups in chitosan ammonium salts enriched positive charge density of cationic amino groups, and antimicrobial activity of chitosan derivatives, consequently.

Antifungal activity of a series of water soluble chitosan ammonium salts with halogens namely, chitosan-bromoacetate, chitosan-chloroacetate, chitosan-dichloroacetate, chitosan-trichloroacetate and chitosan-trifluoroacetate was evaluated *in vitro* by hypha measurement of three phytopathogens *viz*. *Fusarium oxysporum*, *Phomopsis asparagi* and *Colletotrichum lagenarium*. For example, in case of 1.0 mg/mL of potential antimicrobial agent, and in presence of *F. oxysporum*, inhibitory index values of 55.8%, 69.6%, 71.0%, 73.9% and 78.5% were reported for chitosan-bromoacetate, chitosan-chloroacetate, chitosan-dichloroacetate, chitosan-trichloroacetate and chitosan-trifluoroacetate, respectively. These increased values compared with respect to chitosan (11.8%) were in relation with substitution degree of polysaccharide and protonation degree of amino

groups. As electronegativity of halogenic substituent groups in chitosan ammonium salts decreased in order trifluoromethyl > trichloromethyl > dichloromethyl > chloromethyl > − bromomethyl, the antifungal activity followed same tendency: chitosan-trifluoroacetate > chitosan-trichloroacetate > chitosan-dichloroacetate > chitosan-chloroacetate > chitosan-bromoacetate > chitosan); such that a stronger adherence of antimicrobials to outer membranes of fungi produced more damage to fungal integrity and transport of nutrients was affected (Tan et al. 2016).

Starting from 6-bromo-6-deoxy-*N*-phthaloyl-chitosan, Li et al. (2016) synthesized triazole chitosan quaternary ammonium iodide, chloro-1,2,3-triazole chitosan quaternary ammonium iodide and bromo-1,2,3-triazole chitosan quaternary ammonium iodide. In case of chloro-1,2,3-triazole chitosan quaternary ammonium iodide and bromo-1,2,3-triazole chitosan quaternary ammonium iodide, the growth inhibition of phytopathogens like *F. oxysporum f. sp. niveum* ATCC36116, *F. oxysporum. f. sp. cucumebrium* Owen ATCC42357 and *C. lagenarium* (Pass) Ell.et halst ATCC30016 was due to an interactive effect of halogen and triazole moieties. Chitosan quaternization by chemical grafting reactions with different low molecular weight quaternary ammonium salts represents a usual practice to obtain chitosan derivatives. In this way, limited antibacterial and antifungal activities of chitosan which are related to its cationic character below pH 6.5, were improved. In addition, even solubility of new chitosan derivatives was better compared with pure chitosan.

Later, Overvidez-Munoz et al. (2017) functionalized amine groups of chitosan with different quaternary ammonium salts such as 4-bromobutyl-benzalkonium bromide, 4-bromobutyl-triethylammonium bromide or 4-bromobutyl-pyridinium bromide, resulting in following derivatives of chitosan: 4-bromobutyl-benzalkonium bromide chitosan, 4-bromobutyl-triethylammonium bromide chitosan and 4-bromobutyl-pyridinium bromide chitosan. Amongst these modified chitosan samples, 4-bromobutyl-pyridinium bromide chitosan showed highest antibacterial activity, while 4-bromobutyl-triethylammonium bromide chitosan was the least active. Chitosan bearing benzalkonium, triethylammonium and pyridinium moieties, resulting from mixing of 4-bromobutyl-benzalkonium bromide, 4-bromobutyl-triethylammonium bromide and 4-bromobutyl-pyridinium bromide in a weight ratio of 1:1:1, was less soluble, and displayed a reduced antimicrobial activity due to an improper contact of insoluble material with bacterial cells.

Wang et al. (2016) intended to modify the hydroxyl group of chitosan from C_2 position, and prepared five water-soluble *O*-quaternary ammonium salt-chitosans bearing *N*-methyl-*N*-R-*N*, *N*-bis(2-hydroxyethyl) ammonium bromides where radical R represented benzyl, dodecyl, tetradecyl, hexadecyl or octadecyl. Because the new amphiphilic chitosan derivatives presented lipotropic character due to long carbon chains, and hydrophilic character due to quaternary ammonium salt moiety within *O*-quaternary ammonium salt-chitosan backbone, its antibacterial abilities and cytotoxicity were consistently improved compared to their low molecular weight quaternary ammonium salt homologues. In other works, a chitosan quaternary ammonium salt, named *N*-(2-hydroxyl)propyl-3-trimethyl ammonium chitosan chloride, obtained by grafting glycidyl trimethylammonium chloride on chitosan, was the subject of subsequent transformations: combination with

commercial-grad Reactive Red x-3b (Tang et al. 2016) or phthalic anhydride (Chen et al. 2016). Using Reactive Red x-3b, a color reactive bearing sulfonic groups, suitable for textile and wood, a biopolymer dye with improved water solubility and antibacterial properties compared with pure low molecular weight dye was achieved. It was observed that in presence of new biopolymer dye, number of bacterial colony was reduced from 79% to 99% in case of *E. Coli*, and from 73% to 99% in case of *S. aureus* due to a synergic effect of quaternary ammonium and sulfonic groups from structure of biopolymer dye (Tang et al. 2016).

Following esterification reaction of *N*-(2-hydroxyl)propyl-3-trimethyl ammonium chitosan chloride with phthalic anhydride, subsequent introduction of NCO-sulfobetaine onto OH position of *N*-phathaloyl quaternized chitosan derivative were confirmed. Amine groups of resulting *N*-phathaloyl quaternized betainized chitosan were recovered using an aqueous solution of hydrazine monohydrate (Chen et al. 2016). The final zwitterionic product (*O*-sulfobetaine-*N*-(2-hydroxyl)propyl-3-trimethyl ammonium chitosan chloride) fulfilled role of materials with enhanced antibacterial activity and biocompatibility based on synergic action of sulfobetaine and quaternary ammonium groups. Improved water solubility, low cytotoxicity and hemolytic activity of zwitterionic chitosan derivatives are among the necessary parameters for their application under *in vitro* and *in vivo* condition. Beside sulfonic and sulfobetaine groups, thiourea may synergistically act, potentiating the antimicrobial properties of the compound carrying quaternary ammonium groups. Usually, benzoyl thiourea and acyl thiourea derivatives of chitosan display antifungal activity (Eweis et al. 2006; Mohamed and El-Ghany 2012). Earlier, Li et al. (2015) introduced double antibacterial groups, namely *O*-quaternary ammonium and *N*-acyl thiourea, onto OH and NH_2 positions of macromolecular chains of chitosan following few steps: synthesis of *N*-benzylidene-chitosan; quaternization of *N*-benzylidene-chitosan with glycidyl trimethylammonium chloride resulting in *O*-quaternary ammonium *N*-benzylidenechitosan; synthesis of *O*-quaternary ammonium chitosan; reaction of acyl thiocyanate with *O*-quaternary ammonium chitosan resulting in *O*-quaternary ammonium-*N*-acyl thiourea chitosan. In case of *S. aureus*, *Bacillus subtilis, E. coli*, *Pseudomonas aeruginosa* and *Aspergillus niger*, antibacterial activity increased in order: *O*-quaternary ammonium-*N*-acyl thiourea chitosan > *O*-quaternary ammonium chitosan > chitosan. Zeta potential measurements and transmission electron microscopy images confirmed hypothesis related to electrostatic interactions between cationic amino groups and negative charged cell membranes. Thus, higher values of zeta potential in case of quaternized chitosans compared with pure chitosan indicated an increase in positive charge of chitosan derivatives and an increased number of electrostatic interactions. Also, changes in bacterial morphology after treatment with *O*-quaternary ammonium-*N*-acyl thiourea chitosan were due to the rupture of cell membranes and modification of cell metabolism as a result of quaternized chitosan infiltration.

Knowing that chitosan quaternary ammonium salt has antimicrobial and good antioxidant properties, Song et al. (2016) attempted to integrate it in a traditional dental material in order to form a resin base which could improve the overall oral health. They used *N*-trimethyl ammmonium chitosan chloride, denture water

(containing methyl methacrylate monomer, cross-linking agent, inhibitor and an UV absorber) and denture powder (containing poly(methyl methacrylate)), and prepared two types of formulations in accordance with order of material mixing. Material 1 was prepared physically by adding varying amount of chitosan quaternary ammonium salt directly to denture powder, and then denture water. Material 2 was prepared chemically by adding variable amount of *N*-trimethyl ammmonium chitosan chloride to denture water, which determined the graft copolymerization of methyl methacrylate monomer with the chitosan quaternary ammonium salt. Both formulations presented low cytotoxicity and insignificantly modified tensile strength. In contrast, these materials recorded different antimicrobial properties and degrees of corrosion resistance. Antimicrobial tests were carried out with microorganisms specific to oral cavity: *Streptococcus mutans* and *Candida albicans*. In case of both materials, antibacterial rate increased with concentration of chitosan quaternary ammonium salt. As material 1 may have higher specific surface area over chemically produced material 2; it recorded a better performance in both antibacterial and antifungal tests as compared to material 2. In addition, material 2 did not recorded any antifungal property.

5.3.3 Dextran Type Antimicrobials

Naturally synthesized by *Leuconostoc mesenteroides* and *S. mutans* bacteria, dextrans are complex glucans composed of glucose molecules linked by α-1,6 glycosidic linkages in main chain or by α-1,3 glycosidic linkages in branching points. These polysaccharides with various molecular weights due to branched glucose chains of variable lengths are intensively used in medicine and laboratory as anticoagulants, size-exclusion chromatography matrices, cell osmotic pressure or blood sugar level regulators, and components of biosensors or bioreactors. To diversify its degree of application, dextran could be chemically modified such that to obtain cationic amphiphilic dextran derivatives with potential application as broad spectrum external biocides.

Quaternary ammonium groups were attached to dextran main chain bearing alkyl or dialkyl end groups by reaction of modified polysaccharide with an equimolar mixture of a tertiary amine (*N,N*-dimethyl-*N*-benzylamine, *N,N*-dimethyl-*N*-octylamine or 1-methylimidazol) and epichlorohydrin. Obtained cationic amphiphilic dextran derivatives were tested from microbiological point of view (Tuchilus et al. 2017). It was observed that all samples were ineffective against *P. aeruginosa* ATCC 27853, but displayed antimicrobial activity against *E. coli* ATCC 25922, *S. aureus* ATCC 25923, *Sarcina lutea* ATCC 9341, *Candida glabrata* ATCC MYA2950, *C. albicans* ATCC 90028 and *C. parapsilosis* ATCC 22019. Besides type of microbes, antimicrobial activity of samples was correlated with polymer chemical composition. Thus, self-assembling properties, and consequently interactions with microbial cellular components, were tuned by dextran with relative molar mass of 6000 or 10,000, chemical structure of ammonium groups e.g. R_3 = benzyl,

octyl; R_2 = dimetyl-R_3-quaternary ammonium, imidazol; R_1 = dodecyl, octadecyl, and length of end alkyl group, meaning by hydrophilic/hydrophobic balance. Even that length of alkyl or dialkyl chains like C_{12}, C_{18} or $(C_{12})_2$ did not significantly influenced antimicrobial activity of samples originating from dextran with the same molecular mass, modified polymers based on dextran 10,000 recorded lower activity compared with those coming from dextran 6000. In addition, polymers with *N,N*-dimetyl-*N*-benzyl-quaternary ammonium groups showed the best results in case of all tested microorganisms, while samples with *N,N*-dimetyl-*N*-octyl-quaternary ammonium groups have a preference for *E. coli*, and imidazolium based polymers induced only a weak antifungal activity.

5.3.4 Pullulan Type Antimicrobials

Pullulan is a neutral homopolysaccharide with linear chain consisting of almost regularly repeating α-(1 → 4)–maltotriosyl units (3-D-glucopyranosyl) joined through α-(1 → 6) linkages. It looks like an amorphous slime formed by aerobic fermentation of *Aureobasidium pullulans* polymorphic fungus (Singh et al. 2008; Cheng et al. 2011). Molecular weight of this exopolysaccharide is from 4.5×10^4 to 6×10^5 Da and varies with yeast cultivation parameters. Hydroxyl functional groups as a part of flexible polysaccharide chain are the subject for chemical modifications and can result into a series of derivatives: pullulan succinylate, pullulan acetate, pullulan amine, carboxymethyl pullulan, cholesterol bearing pullulan (Singh et al. 2015). In this way, chemical and physical properties of pullulan such as hygroscopicity, biodegradability, viscosity, non-reducing agent, water solubility, formation of oxygen impermeable films are improved due to solubility in organic solvents or enrichment with new reactive functional groups.

Since its introduction as food additive by the Japanese, pullulan and its derivatives recorded numerous applications in foods, pharmaceutical and medical industries. Exploiting antimicrobial potential of quaternary ammonium groups, Grigoras et al. (2013) designed three pullulan derivatives. Length and frequency of grafts influenced all solutions properties, and consequently antimicrobial activity. Diameter of inhibition zones, recorded in presence of *S. aureus* ATCC 25923 strains and salted aqueous polymer solutions, varied from 7 mm to 10 mm in accordance with solution concentration and poly(3-acrylamidopropyl) trimethylammonium chloride content in grafted polymer. Antibacterial activity of grafted pullulans was due to electrostatic interactions between quaternary ammonium cation of poly(3-acrylamidopropyl)trimethylammonium chloride and phosphatidylethanolamine, a negatively charged component from bacterial cell wall.

5.3.5 Starch Type Antimicrobials

Starch (amylum) is a polysaccharide synthesized by plants and structurally formed from amylase and amylopectin molecules in variable proportions. Because of their widespread availability, biodegradability and environmental friendliness, this macromolecule and its derivatives frequently are used as flocculants and disinfectants in water treatment industry. Cationic starch based flocculants possessing quaternary ammonium functional groups on backbone have been developed by grafting reaction or esterification, a more convenient, cost saving, and reliable reaction. Huang et al. (2017) synthesized four different starch-graft-poly(2-methacryloyloxyethyl) trimethyl ammonium chlorides with various grafting ratios, while Liu et al. (2017) reported synthesis of four starch-3-chloro-2-hydroxypropyl triethylammonium chlorides having various quaternary ammonium salt groups on starch backbone. Tests on synthetic turbid and microbiological contaminated waters revealed that starch based derivatives have dual functionality: flocculation effect and antimicrobial performance. Three dimensional excitation emission matrix fluorescence *spectra* of supernatants of bacterial cultures and scanning electron microscopy images of *E. coli* CMCC 44102 and *S. aureus* CMCC 26003 before and after flocculation using cationic starch based flocculants are used to represent antibacterial properties of these starch derivatives. Fluorescence signals from wavelength intervals of 200–240 nm and 280–380 nm, attributed to protein like extracellular organic matters, were more intense for *E. coli* than for *S. aureus* suggesting the higher breakdown of *E. coli* cells. Also, after flocculation, treatment with cationic starch based derivatives, changed shape of bacteria from uniform elliptical to anamorphic one in case of *E. coli*, while most of *S. aureus* cells preserved their spherical shape or appeared "pinched". The study suggested that due to synergistic effect from inorganic particles of kaolin and cationic starch based flocculants, bacterial cell walls were effectively destroyed through strong electrostatic interactions.

5.3.6 Cashew Gum Type Antimicrobials

Cashew gum is a chemical constituent of *Anacardium occidentale* L. tree stem, extracted in order to prepare viscous and emulsifying suspensions for food and pharmaceutical industries. This anionic heteropolysaccharide is composed of β-D-galactose, α-D-glucose, arabinose, rhamnose and glucuronic acid in proportion of 72%, 14%, 4.6%, 3.2% and 4.6%, respectively. Galactose units are joined by β $(1 \rightarrow 3)$ links in main chain of macromolecule or by β $(1 \rightarrow 6)$ links in side chain (de Paula et al. 1998). To improve its weak antimicrobial activity (Campos et al. 2012), Quelemes et al. (2017) chemically modified cashew gum by quaternization reaction. Firstly, cashew gum alkoxide was reacted with epoxide- or

N-(3-chloro-2-hydrohypropyl) trimethylammonium chloride. In this way, alkoxide groups of cashew gum were nucleophilic substituted by cationic quaternary ammonium moieties. All three resulted quaternized cashew gum derivatives with degree of substitution of 42%, 68% and 73% were tested against eleven strains of *Staphylococcus* genus. Generally, it was observed that minimum inhibitory concentration and minimum bactericidal concentration values decreased with the increase in degree of substitution; anti-staphylococcal activity was enhanced due to more incorporated positive charges in quaternized cashew gum. Influence of the most promising derivative on bacterial morphology was evaluated using atomic force microscopy. After a 24 h exposure of methicilin sensitive *S. aureus* ATCC 29213 culture to solution of quaternized cashew gum having 73% degree of substitution, mean height of cells changed from 0.9 μm (in case of untreated cultures) to 1.4 μm or 1.3 μm at minimum inhibitory concentration or minimum bactericidal concentration. In addition, integrity of cell wall was gradually affected due to partial damage of bacterial cell. Also, all quaternized cashew gum derivatives showed good biocompatibility on erythrocytes, fibroblasts and keratinocytes which recommended them as promising agents against skin pathogens.

5.3.7 Poly(lactide) Type Antimicrobials

Being a non-toxic, biodegradable and bioactive bioplastic intensively used globally, poly(lactic acid) or poly(lactide) represents a thermoplastic aliphatic polyesther extracted from natural sources like sugarcane, corn starch or cassava roots, and used as fibers or films. Thus, poly(lactide) fibers could be more sustainable compared with other polyesther fibers derived from petroleum.

Usually, finishing of poly(lactide) fibers is carried out by their treatment with an antimicrobial film of silver or zinc oxide nanoparticles, essential oils, antibiotics or chitosan. Some of these antimicrobial coatings operate by mechanism of controlled release or by formation of a biological barrier. To increase the effectiveness of antimicrobial protection in case of poly(lactide) fibers, Logar et al. (2016) tailored antimicrobial coatings with dual and synergistic activities using silver and quaternary ammonium functional groups. They developed a three-stage finishing procedure which involved creation of a silica matrix (I), in situ synthesis of AgCl from silver nitrate and sodium chloride (II) and in third stage, application of silver chloride and 3-(trimethoxysilyl)-propyldimethyltetradecyl ammonium chloride. Even though application of this coating generated some esthetic disadvantages for poly(lactide) fibers such as reduced lightness and increased yellowing; from microbiological point of view it displayed excellent bactericidal activity with a 99.99% reduction in *S. aureus* and *E. coli* colonies.

A water soluble cationic benzanthrone derivative namely 1-[(7-oxo-7H-benzo[de]anthracen-3-ylcarbamoyl)-methyl]-triethylammonium chloride was

synthesized and incorporated into a poly(lactide) thin film in order to test its antimicrobial activity (Staneva et al. 2015). The compound was observed to be very photostable and exhibited a prolonged release of benzanthrone derivative into aqueous solution. The system composed of poly(lactide) matrix and benzanthrone derivative was tested against a wide spectra of microorganisms (*Bacillus cereus*, *B. subtilis*, *S. lutea*, *Micrococcus luteus*, *E. coli*, *Pseudomonas aeruginosa*, *Acinetobacter johnsonii*, *S. cerevisiae*, *Xanthomonas oryzae*, *Candida lipolytica*), and minimum inhibitory concentration was in range of 48–125 μg/mL and zones of inhibition were in range of 12–24 mm. Also, optical densities of growth media for *E. coli* and *P. aeruginosa* strains decreased in the presence of composite material compared with untreated poly(lactide) matrix. Authors attributed the antimicrobial activity of new system to action of cationic quaternary ammonium groups onto plasma membrane of yeasts and cytoplasmic (inner) membrane of bacteria.

5.4 Antimicrobials Based on Synthetic Polymers

Because natural sources are limited, sometimes researchers attempt to design synthetic polymers with antimicrobial activities. Biocompatible synthetic polymers like poly(amidoamine), poly(urethane)s, poly(siloxane)s, poly(methacrylate)s and poly(ethylene) have found applications in various medical subdomains. In addition, these have efficient antimicrobial properties and are non-toxic for rest of cells, too.

5.4.1 Poly(amidoamine) Type Antimicrobials

A new trend to enhance the contact surface between pathogenic cells and antimicrobial substances involves the use of tree like branched polymers, with a sphere like shape, named dendrimers. Modulating synthesis conditions of these molecules, the number of branches could exponentially increase with each new generation of branches. Poly(amidoamine) is a representative of dendrimer class, composed of repetitively branched subunits of amide and amine functionality. Because it is biocompatible, easy to synthesize and has similar properties with globular proteins, some researchers had functionalized this macromolecule in order to fabricate novel antibacterial agent for water treatment. Thus, Maleki et al. (2017) modified poly(amidoamine) dendrimers (generations G2 and G4) into quaternary ammonium salts using Cl, Br, I halogens groups. Then, antimicrobial activity was determined against *E. coli*, *Klebsiella oxytoca*, *B. subtilis*, and *S. aureus* isolated from contaminated water samples. Compared with unmodified polymers, quaternary ammonium salts exhibited an enhanced antimicrobial efficacy against bacteria, the most effective being G4 generation of poly(amidoamine) modified with iodine.

5.4.2 Poly(urethane) Type Antimicrobials

Versatile materials like poly(urethane)s, intensively applied in medical and automotive fields as biomaterials or coatings, are synthesized in forms of foams, starting from di- or triisocyanates and polyols. To avoid potential leaching of physically bonded biocides from polyurethane matrix, some authors chosed chemically bonded cationic groups like imidazolium, pyrrolidonium or ammonium to design antimicrobials with prolonged usage. Thus, Udabe et al. (2017) prepared porous materials with antibacterial properties using glycerol propoxilate, tri- or tetra-hydroxyl functionalized quaternary ammonium compounds, and poly(hexamethylene diisocyanate) or toluene diisocyanate in different compositions. They observed that poly(urethane) ability to grow was enhanced in accordance with amount of quaternary ammonium compound in foam formulation. Also, 20% of quaternary ammonium component in poly(urethane) foams ensured a high antimicrobial activity. Chemically incorporated ammonium groups into poly(urethane) backbone were responsible for killing of *S. aureus* ATCC 29737 and *E. coli* ATCC 25922. In case of *E. coli*, an efficiency of 89% was observed for poly(urethane) formulation with 16% quaternary ammonium content. However, in case of *S. aureus*, maximum killing efficiency was observed with 7% of quaternary ammonium content. Besides, different chemical composition of microorganism structure and different content of reactive antimicrobial groups, type of isocyanate was another influencing factor for antibacterial behavior. In comparison with poly(hexamethylene diisocyanate), the more hydrophobic molecule toluene diisocyanate ensured a higher antimicrobial rate.

In another study, dressings based on poly(urethane) foams containing poly(diallyldimethylammonium chloride), known as polyquaternium-6, were applied on wounds infected with different microorganisms: *S. aureus* AH133; *P. aeruginosa* strain PAO1 Lux; a clinical isolate of *Acinetobacter baumannii*; *S. aureus* Lux; MRSA strains of *S. aureus* (Tran et al. 2017). Wound dressing material was placed on back of laboratory animals and bacterial biofilm formation in tissue under bandage was evaluated. It was observed that poly(diallyl-dimethylammonium chloride) containing poly(urethane) dressings totally inhibited biofilm formation, for all test organisms, reducing wound overinfection.

To endow them with antimicrobial properties, the biodegradable and biocompatible poly(urethane) coatings were successfully prepared by Liu et al. (2015) starting from a mixture of terpene-based polyol, various quaternary ammonium salts and hydrophilically modified hexamethylene diisocyanate tripolymer. In this case, quaternary ammonium salts were synthesized by quaternization reaction of terpene-based carbamate with alkyl bromides. Beside excellent mechanical properties and water resistance, resulted products recorded considerable antimicrobial activities against both *E. coli* and *S. aureus* even at reduced concentrations (5 wt%). As expected, antimicrobial activity of poly(urethane) coatings was improved by introduction of quaternary ammonium groups.

In order to prevent damage of medical devices covered by antimicrobials, but subsequently overpopulated with dead microorganisms, a research team designed a complex reverse surface structure, composed of an contact-active antibacterial upper-layer and a long-lasting antifouling sub-layer (He et al. 2016). Thus, gemini

quaternary ammonium salt waterborne poly(urethane) films casted from solutions have been prepared using: isophorone diisocyanate, poly(tetramethylene glycol), poly(ethylene glycol), *N*,*N*,*N*′,*N*′-tetramethyl-*N*,*N*′-bisdodecyl-2,6-bis(ammonium bromide)–*L*–lysine-(1′,3′-propylene diamide)-*L*-lysine, and *L*-lysine. Poly(tetramethylene glycol) soft segment of modified poly(urethane) films and isophorone diisocyanate were hydrophobic, while soft segment poly(ethylene glycol) and *L*-lysine formed anti-fouling sub-layer, and *N*,*N*,*N*′,*N*′-tetramethyl-*N*,*N*′-bisdodecyl-2,6-bis(ammonium bromide)–*L*–lysine-(1′,3′-propylene diamide)-*L*-lysine antibacterial brushes formed upper layer. By varying molar fraction of components, different gemini quaternary ammonium salt waterborne poly(urethane) samples were obtained. Killing efficiency of this novel surface structure was tested against both types of Gram bacteria. It was observed that after a critical concentration of *N*,*N*,*N*′,*N*′-tetramethyl-*N*,*N*′-bisdodecyl-2,6-bis(ammonium bromide)–*L*–lysine-(1′,3′-propylene diamide)-*L*-lysine (4.96 wt%), all gemini quaternary ammonium salt waterborne poly(urethane) samples displayed strong antibacterial activity. Simultaneously, antifouling properties were related with surface hydrophilicity determined by contact angle measurements. Decreasing of time related water contact angle and enhancing of hydrophilicity at the same time with increase in *N*,*N*,*N*′,*N*′-tetramethyl-*N*,*N*′-bisdodecyl-2,6-bis(ammonium bromide)–*L*–lysine-(1′,3′-propylene diamide)-*L*-lysine, were attributed to the cationic nature of gemini quaternary ammonium fragments from modified poly(urethane)s.

5.4.3 Poly(siloxane) Type Antimicrobials

Poly(siloxane)s, generically called silicones, are biocompatible and biodurable materials widely applied in healthcare industry. State of aggregation of final products could be tuned by varying –Si–O– chain length, side organic groups attached to silicon atoms and crosslinking degree. Thus, silicones with properties characteristic to liquids or hard plastics such as thermal and chemical stability, hydrophobicity or low surface tension, represented matrix for various medical equipment and instruments. When the latter are to be used for therapeutic purposes, conditions of sterility and asepsis should also be considered. In this regard, antimicrobial materials based on silicones and quaternary ammonium moieties were designed.

Starting from poly(siloxane)s containing tertiary amino group and epichlorohydrin, Cui et al. (2015) obtained poly(siloxane) quaternary ammonium salts containing epoxy group with different molecular weights (M_w = 3150–13,000 g/mol) by a quaternization reaction in ethanol. It was observed that the new products spontaneously formed micelles in aqueous solutions and exhibited surfactant properties, as well as thermal and chemical stabilies. From microbiological point of view, poly(siloxane) quaternary ammonium salts containing epoxy group were active against *S. aureus*, *B. subtilis*, and *E. coli* such that increased molecular weight of polymers led to a high antibacterial activity. In addition, sensitivity of microorganisms in relation to poly(siloxane) quaternary ammonium salts containing epoxy

group decreased in order: *B. subtilis* (most sensitive) > *S. aureus* (less sensitive) > *E. coli* (most insensitive).

Beside better wetting, emulsifying and solubilization properties, and lower critical micelle concentration, compared with conventional surfactants, gemini surfactants (molecules composed of two hydrophilic headgroups and two hydrophobic tails connected through a spacer unit) could have antimicrobial properties, especially when they have cationic or zwitterionic character. Taking into account all these advantages, Bao et al. (2017) synthesized some cationic gemini surfactants with poly(ether siloxane) linked group (C_m-PSi-C_m, m = 8, 10, 12, 14, 16, 18). Qualitative and quantitative antimicrobial tests revealed that C_{10}-PSi-C_{10} polymeric surfactants have strongest antimicrobial ability against *E. coli* and *S. aureus*, while C_{14}-PSi-C_{14} possessed the strongest antimicrobial activity against *A. flavus*. As expected, antimicrobial activities of C_8-PSi-C_8 and C_{18}-PSi-C_{18} compounds were insignificant, because the inadequate length of hydrophobic chain (too long or too short) from structure of polymeric gemini surfactants was unfavorable to interactions with tested pathogenic microorganisms.

Another approach to improve antimicrobial properties of biocompatible synthetic polymers involves synthesis of copolymers, especially block copolymers. Because pure poly(dimethylsiloxane) has tendency to form a brittle silica like layer, it was chosen as candidate for blending with antimicrobial agents and subsequently chemical surface modifications. Thus, Qin et al. (2015) designed an antibacterial coating from block copolymers of poly(dimethylsiloxane) with quaternized poly(*N,N*-dimethylaminoethyl methacrylate). In this study, hydrophilic poly(*N,N*-dimethylaminoethyl methacrylate) was converted into cationic form by quaternization of tertiary amino groups with n-octyliodide. Spin coated copolymer films were obtained from acetonitrile solution of block copolymers mixed with hexamethylene diisocyanate. A different chemical composition of quaternized block copolymers was related with variable Si/N ratio: 1.5/1, 0.6/1, 0.7/1 and 1.1/1. In case of some films, mobility of poly(dimethylsiloxane) chains and intermolecular ionic interactions between alkyl side chains and positively charged groups in quaternary ammonium salts have favored appearance of microphase separations. So, surface roughness determined interaction with bacteria such that heterogeneous surfaces ensured an increased contact area with microorganisms and improved antimicrobial activity. From entire series of quaternized block copolymers, the exponent with the higher content of N^+ showed antimicrobial activity towards both *B. subtilis* and *E. coli*. In contrast, the other three were insensitive towards Gram negative bacteria.

Self-assembling capacity of block copolymers was exploited by Zhou et al. (2015) in order to design new fluorosilicone copolymers containing bloks of dithiocarbonate terminated poly(dimethylsiloxane), poly(2-(dimethylamino)ethyl methacrylate) quaternized with n-octyliodide, poly(hexafluorobutyl methacrylate) and hydroxyethyl methacrylate, respectively. The new quaternized multi block copolymers contained different mass percentages of poly(2-(dimethylamino)ethyl methacrylate) and poly(hexafluorobutyl methacrylate). Visualization of inhibition zones around multi block copolymer films, in presence of representative Gram positive bacteria (*B. subtilis* ATCC 63501) and Gram negative bacteria (*E. coli* ATCC 44752), helped to correlate antimicrobial activities of these quaternized multi block

copolymers with the stronger tendency of poly(dimethylsiloxane) and quaternary ammonium salts related blocks to migrate to surface of films, compared with poly(hexafluorobutyl methacrylate) blocks. Thus, fluorosilicone multi block copolymer containing appropiate amount of poly(hexafluorobutyl methacrylate), usually lower than 26.1 wt%, recorded antibacterial activity due to a higher C-N^+ content and ability to form films with relatively smooth morphology.

5.4.4 Poly(methacrylate) Type Antimicrobials

Monomethacrylates and di-methacrylates are monomers intesivelly used in polymerization reactions resulting in thermoplastic materials with excellent weatherability, good mechanical properties and chemical resistance. Makvandi et al. (2018) reviewed antibacterial properties of dental composite materials composed of a quaternary ammonium modified methacrylate resin matrix, beside inorganic microfillers or nanofillers, coupling agents, and an initiator–accelerator system. Even that dental restoration materials incorporated with quaternary ammonium compounds exert a long time antibacterial effect compared with other antimicrobials, e.g. silver or zinc oxide nanoparticles, a balance between antimicrobial properties and mammalian cell cytotoxicity must always be considered.

Lv and team (2018) designed an electrochemical biosensor for immobilization of redox protein, which possesed antimicrobial properties, too. They used positively charged polymeric quaternary ammonium salts of dimethylaminoethyl methacrylate and negatively charged hemoglobin for constructing a film onto a glassy carbon electrode. Catalytic activity of immobilized hemoglobin was evaluated toward peroxide of hydrogen, and antimicrobial properties were tested against *E. coli* (ATCC 25922), *S. aureus* (ATCC 6538), *Candida albicans* (ATCC 14053) and *Aspergillus fumigatus* (Af 293). Both properties were influenced by the length of alkyl chain or type of methacrylate molecule (macromolecule or monomer).

5.4.5 Poly(ethylene) Type Antimicrobials

Beside well-known plastics like poly(urethane), poly(styrene), poly(propylene) or poly(vinyl chloride), being intensively used in daily life, poly(ethylene) is another thermoplastic semicrystalline polymer usually applied in packaging industry or medical instruments and devices. Because most of poly(ethylene) type materials come into contact with living beings, it is necessary to ensure their sterility. Thus, Rossetti et al. (2017) mixed poly(ethylene) from melt with mono- and bicationic quaternary ammonium salts. They used nine different salts: benzyldimethylstearylammonium chloride; trimethylstearylammonium chloride; dimethyldistearylammonium chloride; methyltristearylammonium bromide; tetrastearylammonium bromide; 1,12-bis(dimethyloctylammonium)dodecane dibromide; 1,20-bis(dimethyloctylammonium)eicosane dibromide; 1,30-bis(dimethyloctylammonium)

triacontane dibromide; bis(dimethyloctylammonium)PEG(400) ditosylate. Resulted antimicrobial poly(ethylene)s were self-organized in such a way that quaternary ammonium salts bounded by Vander-Waals forces were displayed to the surface of polymer, forming nonleaching, permanent biocidal surfaces.

5.5 Target Microorganisms Affected by Polymeric Antimicrobials Containing Quaternary Ammonium Moieties

Eradication of various pathogens such as viruses, bacteria, fungi, algae from surrounding world, or of opportunistic germs from living organisms, involves the use of more or less aggressive physical or chemical methods. Each method is chosen according to the main structural features of target microorganism. In this regard, it takes in consideration hydrophilic character of Gram positive bacteria or hydrophobic character of Gram negative bacteria. This difference is due to synergic action of main components of cells. Thus, murein layer in Gram positive bacteria cell wall is thick, cross-linked and reinforced with teichoic and lipoteichoic acids, and displayed to external part of cell wall. In return, in case of Gram negative bacteria, thin layer of murein is located in periplasmic space between outer and inner membranes. If membrane of Gram positive bacteria is composed of cardiolipin, predominant phospholipids in inner membrane of Gram-negative bacteria are phospahtidylethanolamine and phosphatidylglycerol. In addition, outer membrane of Gram negative bacteria is covered with a lypopolysaccharides layer, while in case of Gram positive bacteria, outer membrane is missing. Even chemical structure of fungi is variable. For example, mycelium hyphae consisting of branched filament tubes are surrounded by a rigid cell wall with variable chemical composition, most of fungi containing chitin and glucan. Some fungus like organisms have a cell wall made up of considerable amounts of cellulose, while cell surface of *Candida albicans* is negatively charged due to terminal sialic acid, which is found on the surface of the membrane. There is a wide spectrum of pathogens studied by researchers, but representative microorganisms tested in the presence of polymers containing quaternary ammonium moieties are limited and grouped in Table 5.1 or schematically represented in Fig. 5.1 and Table 5.2, respectively.

A possible lower antimicrobial activity of quaternary ammonium polymers compared with standard antibiotics is balanced by their broader activity. Bacteriostatic/fungistatic and bactericidal/fungicidal effects of quaternary ammonium polymers are related to passive or active mechanism of action of substances in the presence of microorganisms. If a passive action of antimicrobials is supposed to repel pathogenic entity due to hydrophilic/hydrophobic/electrostatic repulsion or low surface energy, an active action involves microorganism killing by electrostatic or biocidal interaction (Huang et al. 2016). In depth, molecular mechanism of antimicrobial action is carried out along several stages: adsorption of antimicrobial agent onto cell surface/to outer membrane; formation of interface complexes between

Table 5.1 Representative microorganisms tested in presence of polymers with quaternary ammonium moieties

Microorganisms	*Species*	References
Gram positive bacteria	*Staphylococcus aureus*, *S. epidermidis*	Meng et al. (2015), Mohamed et al. (2015), Fan et al. (2015), Ignatova et al. (2016), Tang et al. (2015), Oyervides-Munoz et al. (2017), Wang et al. (2016), Tang et al. (2016), Chen et al. (2016), Li et al. (2015), Tuchilus et al. (2017), Grigoras et al. (2013), Quelemes et al. (2017), Logar et al. (2016), Maleki et al. (2017), Udabe et al. (2017), Liu et al. (2015), He et al. (2016), Cui et al. (2015), Bao et al. (2017), Lv et al. (2018), Rossetti et al. (2017)
	Streptococcus mutans	Song et al. (2016)
	α-Hemolytic Streptococcus	Wang et al. (2016)
	β-Hemolytic Streptococcus	Wang et al. (2016)
	Bacillus subtilis	Li et al. (2015), Maleki et al. (2017), Cui et al. (2015), Qin et al. (2015), Zhou et al. (2015)
	Enterococcus faecalis	Mohamed et al. (2015)
	Sarcina lutea	Tuchilus et al. (2017)
Gram negative bacteria	*Escherichia coli*	Meng et al. (2015), Mohamed et al. (2015), Fan et al. (2015), Ignatova et al. (2016), Tang et al. (2015), Oyervides-Munoz et al. (2017), Wang et al. (2016), Tang et al. (2016), Chen et al. (2016), Li et al. (2015), Tuchilus et al. (2017), Logar et al. (2016), Staneva et al. (2015), Maleki et al. (2017), Udabe et al. (2017), Liu et al. (2015), He et al. (2016), Cui et al. (2015), Bao et al. (2017), Qin et al. (2015), Zhou et al. (2015), Lv et al. (2018), Rossetti et al. (2017)
	Pseudomonas aeruginosa	Wang et al. (2016), Li et al. (2015), Tuchilus et al. (2017), Staneva et al. (2015), Rossetti et al. (2017)
	Proteus vulgaris	Wang et al. (2016)
	Klebsiella pneumoniae, K. oxytoca	Mohamed et al. (2015), Maleki et al. (2017)
Fungi	*Saccharomyces cerevisiae*	Peng et al. (2016)
	Aspergillus niger, *A. fumigatus*, *A. flavus*	Mohamed et al. (2015), Wang et al. (2016), Li et al. (2015), Bao et al. (2017), Lv et al. (2018)
	Candida albicans, *C. glabrata*, *C. parapsilosis*	Wang et al. (2016), Song et al. (2016), Tuchilus et al. (2017), Lv et al. (2018)
	Colletotrichum lagenarium (Pass) Ell. et halst	Tan et al. (2016), Li et al. (2016)
	Fusarium oxysporum f.sp.niveum	Tan et al. (2016), Li et al. (2016)
	Fusarium oxysporum.f.sp. cucumebrium Owen	Tan et al. (2016), Li et al. (2016)
	Phomopsis asparagi	Tan et al. (2016)
	Geotricum candidum	Mohamed et al. (2015)

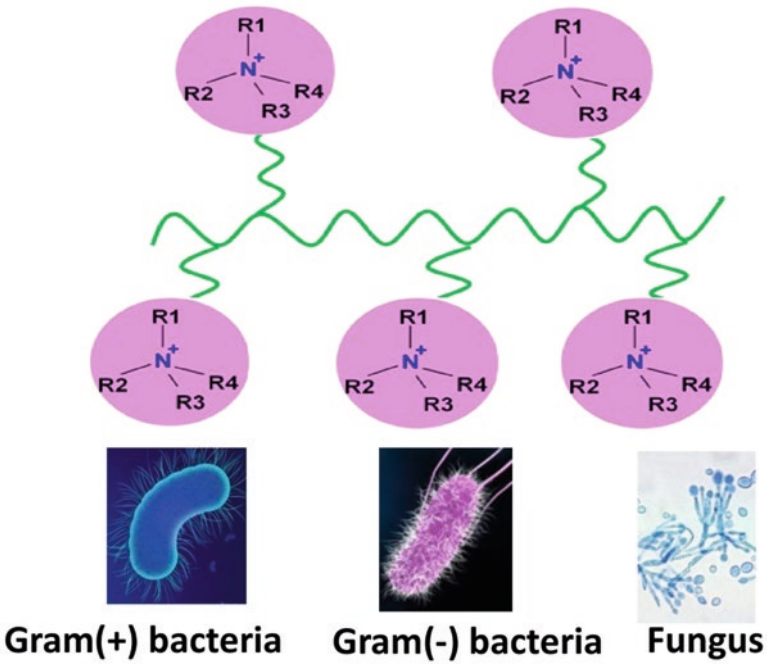
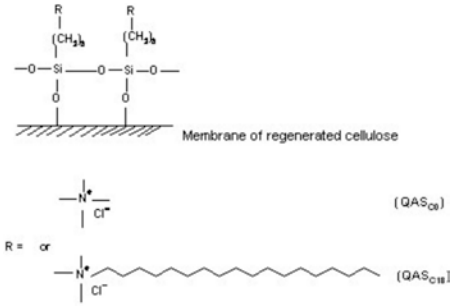
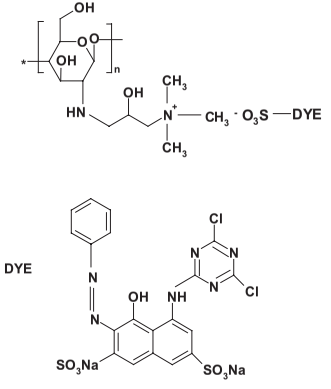


Fig. 5.1 Schematic representation of microorganisms and polymer chain bearing quaternary ammonium moieties. Under favorable conditions, macromolecular chains bearing positive charged groups of quaternary ammonium can interact with components of microbial entities

microorganism membrane and antimicrobial substance by diffusion through cell wall and binding of substances to cytoplasmic membrane; cytoplasmic membrane disruption; translocation of negatively charged lipids of membrane from inside part to outside part of membrane and lateral segregation of membrane lipids; destabilization of outer membrane by releasing cytoplasmic constituents like potassium ions, DNA and RNA; interaction of genetic material with biocidal agent; cell death (Munoz-Bonilla and Fernandes-Garcia 2012; Timofeeva and Kleshcheva 2011).

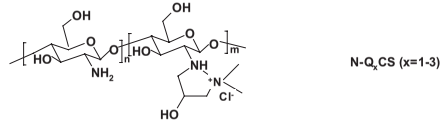
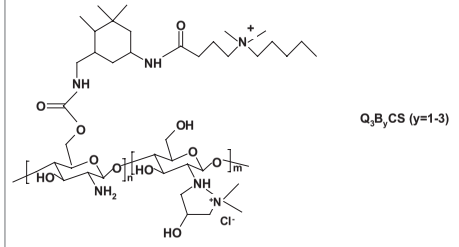
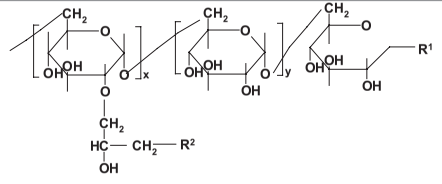
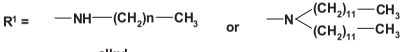
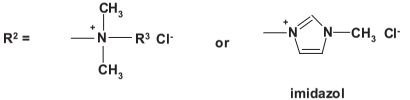
Preliminary qualitative microbiological tests and extensive quantitative microbiological methods are used in order to evaluate antimicrobial activity of substances. Some of parameters directly refer to microorganism number affected by potential antimicrobials; others are indirectly related with the effect of antimicrobials. Usually, colony forming units are counted, while cell viability, inhibitory index, bacterial reduction, and antimicrobial efficiency are assessed in percentage. Other parameters are also estimated from microbiological tests: bactericidal activity in time (h), bacteriostatic efficiency (log reduction), minimum inhibitory concentration, minimum bactericidal concentration and minimum fungicidal concentration. There are different approaches to evaluate inhibition zone; some authors include in diameter of zone of inhibition, even the size of cylinder or disk containing or immersed with antimicrobial solution; while others calculate zone of inhibition (in mm) by separating support “imprinted” with antimicrobial solution from diameter of transparent zone. Table 5.3 presents the antimicrobial activity of polymers

Table 5.2 Chemical formulas of polymers with quaternary ammonium moieties and antimicrobial activity (selected from literature)

Quaternary ammonium polymers	Chemical formulas	References
Regenerated cellulose (RC) membrane surface croslinked with trimethoxysilylpropyl trimethyl ammonium chloride ($QASC_0$) or trimethoxysilylpropyl octadecyldimethyl ammonium chloride ($QASC_{18}$)		Meng et al. (2015)
Chitosan reacted with with glycidyl trimethylammonium chloride and dye		Tang et al. (2015)

(continued)

Table 5.2 (continued)

Quaternary ammonium polymers	Chemical formulas	References
N-(2-hydroxyl)propyl-3-trimethyl ammonium chitosan *N*-QxCS (x = 1–3) as of N-quaternary chitosan derivatives *O*-sulfobetaine-*N*-(2-hydroxyl)propyl-3-trimethyl ammonium chitosan chloride Q3ByCS (y = 1–3) as zwitterionic chitosan derivatives	 N-Q$_x$CS (x=1-3)  Q$_3$B$_y$CS (y=1-3)	Chen et al. (2016)
Cationic amphiphilic dextran derivatives	  alkyl dialkyl  imidazol R^3 = benzyl, octyl R^2 = dimetyl-R3-quaternary ammonium, imidazol R^1 = dodecyl, octadecyl	Tuchilus et al. (2017)

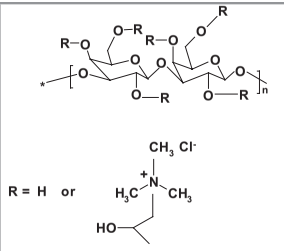
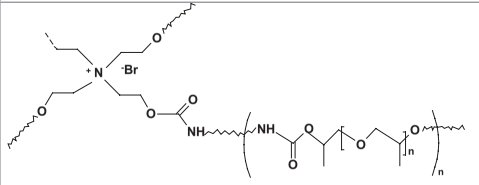
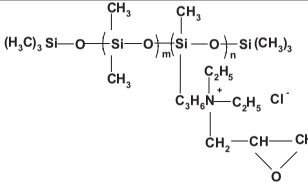
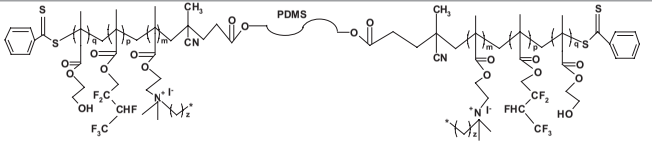
Cashew gum quaternized with (3-chloro-2-hydroxypropyl) trimethylammonium chloride		Quelemes et al. (2017)
Polyurethane bearing *N*-tetraethanolammonium bromide		Udabe et al. (2017)
Poly(siloxane) quaternary ammonium salts containing epoxy group		Cui et al. (2015)
Quaternized multi block copolymers composed of dithiocarbonate-terminated poly(dimethylsiloxane) PDMS, poly(2-(dimethylamino)ethyl methacrylate) quaternized with n-octyliodide, poly(hexafluorobutyl methacrylate) and hydroxyethyl methacrylate		Zhou et al. (2015)

Table 5.3 Antimicrobial activity of polymers with quaternary ammonium moieties

Polymeric antimicrobials	Tested microorganisms	Microbiological parameters/tests			References
Natural polymers					
RC-QAS$_{(C0)}$ membrane	*E. coli* (DH5α)				Meng et al. (2015)
	S. aureus (ATCC 6538)	Cell viability (%)			
			RC-QAS$_{(C0)}$	RC-QAS$_{(C18)}$	
RC-QAS$_{(C18)}$ membrane	Original inoculum:				
	1× 10^6 CFU/mL	*E. coli*	~99.9	0.4	
		S. aureus	~99.9	0.5	
Hydrogels containing quaternized cellulose (QC) and native cellulose:	*Saccharomyces cerevisiae* N85	Cell viability (%)			Peng et al. (2016)
	Original inoculum:	Gel9-2	88		
	10^6–10^7CFU/mL	Gel9-4	33.3		
		Gel9-6	33.6		
Gel9-2 (D.S. 0.23)		Gel25-2	87.1		
Gel9-4 (D.S. 0.42)		Gel25-4	66.9		
Gel9-6 (D.S. 0.61)		Gel25-6	19.9		
Gel25-2 (D.S. 0.28)					
Gel25-4 (D.S. 0.44)					
Gel25-6 (D.S. 0.69)					
NQC	S1: *B. subtilis* RCMB 010067	Diameter of inhibition zone (mm)			Mohamed et al. (2015)
Q1P3	S2: *S. aureus* RCMB 010028				

Q1P1	S3: *Klebsiella pneumoniae* RCMB 0010093		NQC	Q1P3	Q1P1	Q3P1	Genta micin	Ampi cilin	Amphotericin B	
Q3P1	S4: *E. coli* RCMB 010052	S1	8	13–17	15–19	12–16	26	–		
	S5: *Aspergillus fumigatus* RCMB 02568	S2	11	15–19	16–20	14–17	22	–		
	S6: *Geotricum candidum* RCMB 05097	S3	11	16–20	17–20	15–18	–	28		
		S4	13	17–22	17–25	16–20	–	32		
		S5	11	13–20	15–20	13–18			24	
		S6	10	13–21	13–23	13–19			29	
HACC/PVA/PEO hydrogel	*S. aureus* RCMB 000106	Diameter of inhibition zone (qualitative test)								Fan et al. (2015)
	E. coli RCMB 000107	*S. aureus*				*E. coli*				
		++				++				
QCh	*S. aureus* 749	Bactericidal activity in time (h)								Ignatova et al. (2016)
Car	*E. coli* 3588 (National Bank for Industrial Microorganisms and Cell Cultures, Sofia, Bulgaria)					*S. aureus*		*E. coli*		
CA/PHB		CA (1800 mg/mL)				24		–		
QCh/Car-coated PHB		CA/PHB				24		24		
QCh/Car-(CA/PHB)		QCh (3000 mg/mL)				1		2		
CA/QCh/Car-PHB		QCh/Car-coated PHB				4		6		
		QCh/ Car-(CA/PHB)				3		4		
		CA/QCh/Car-PHB				3		4		

(continued)

Table 5.3 (continued)

Polymeric antimicrobials	Tested microorganisms	Microbiological parameters/tests				References
CTS	*E. coli*	Diameter of inhibition zone (mm)				Tang et al. (2015)
CTS–CTA	*S. aureus*					
(CTS–CTA)–I_2				*E. coli*	*S. aureus*	
		CTS		0	0	
		CTS–CTA		11	14	
		(CTS–CTA)–I_2		23	23	
CS		Inhibitory index (%) at 1.0 mg/mL polymer				Tan et al. (2016)
CSB	S1: *Fusarium oxysporum*					
CSC			S1	S2	S3	
CSDC	S2: *Colletotrichum lagenarium*					
CSTC		CS	11.8	25	–	
CSTF		CSB	55.8	69.1	–	
	S3: *Phomopsis asparagi*	CSC	69.6	73.5	–	
		CSDC	71	76.5	~ 80	
		CSTC	73.9	77.9	~ 80	
		CSTF	78.5	82.4	~ 80	

CS		Inhibitory index (%) at 1.0 mg/mL polymer					Li et al. (2016)
TCTS	S1:*Colletotrichum lagenarium*						
CTCTS	(Pass) Ell.et halst ATCC30016		S1	S2	S3		
BTCTS							
	S2:*Fusarium oxysporum f.sp. niveum* ATCC 36116	CS	30	24.2	14.7		
		TCTS	85.1	58.5	86.1		
		CTCTS	93.2	84	91.7		
	S3:*Fusarium oxysporum.f.sp. cucumebrium* Owen ATCC 42357	BTCTS	88.1	81.8	91.7		
Cs	*E. coli* 25922	Diameter of inhibition zone (mm)			Minimum inhibitory concentration (μg/mL)		Oyervides-Munoz et al. (2017)
BZK-Cs	*S. aureus* 25923						
TEA-Cs			*E. coli*	*S. aureus*	*E. coli*	*S. aureus*	
PYA-Cs	Original inoculum:						
BTP-Cs	10^8 CFU/mL	Cs	21	21	256	256	
		BZK-Cs	25	24	128	128	
		TEA-Cs	23	22	128	128	
		PYA-Cs	27	26	128	64	
		BTP-Cs	27	24	128	512	

(continued)

Table 5.3 (continued)

Polymeric antimicrobials	Tested microorganisms	Microbiological parameters/tests							References
CS		Diameter of inhibition zone (mm)							Wang et al. (2016)
BNQAS-CS			CS	BNQAS-CS	C12QAS-CS	C14QAS-CS	C16QAS-CS	C18QAS-CS	
C12QAS-CS									
C14QAS-CS	S1: *S. aureus* ATCC 6538	S1	6.5–11	8–14	8–17	8–15	8–14	7–12	
C16QAS-CS	S2: α-*Hemolytic Streptococcus* (α-H-tococcus, CMCC(B) 31005)	S2	8–12	7–11	9–10	8–12	8–15	7–13	
C18QAS-CS	S3: *β-Hemolytic Streptococcus* (β-H-tococcus, ATCC 21059)	S3	–	7–11	10–16	9–14	9–14	9–14	
	S4: *E.coli* ATCC 25922	S4	7–12	7–12	6–11	7–11	7–11	7–11	
	S5: *P. aeruginosa* ATCC 9027	S5	–	–	8–10	7–11	6–10	–	
	S6: *Proteus vulgaris* CMCC(B) 49027	S6	–	–	7–10	8–12	7–9	–	
	S7: *Aspergillus* CMCC(F) 98003	S7	7	8–16	8–16	7–13	8–16	7–13	
	S8: *C. albicans* ATCC 10231	S8	7–9.5	8–12	8–12	10–14	11–15	8–13	
Chitosan biopolymer dye	*E. coli*	Cell viability (%)							Tang et al. (2016)
	S. aureus	*E. coli*		*S. aureus*					
	Original inoculum:	< 0.1 (after 24 h)		< 0.1 (after 24 h)					
	2.3x 10^8 CFU/mL								

CS	*S. aureus* (ATCC 6538)	Diameter of inhibition zone (mm)			Chen et al. (2016)
N-QCS	*E. coli* (ATCC 25922)				
N-Q2CS			*S. aureus*	*E. coli*	
N-Q3CS	Original inoculum:				
Q3BCS	10^8 CFU/mL	CS	6	6	
Q3B2CS		N-QCS	6	6	
Q3B3CS		N-Q2CS	9	10	
		N-Q3CS	15	15	
		Q3BCS	15	16	
		Q3B2CS	17	17	
		Q3B3CS	18	18	
CS	*S. aureus* ATCC 6538	MIC (w/v %) for tested microorganisms			Li et al. (2015)
OQCS	*E. coli* ATCC 8739				
OQCATUCS-1	*A. niger* ATCC 16404	OQCS		0.025–0.06	
OQCATUCS-3	*P. aeruginosa* ATCC 9027				
OQCATUCS-5	*B. subtilis* ATCC 6633	OQCATUCS		0.0125–0.025	
	Original inoculum:				
	10^8 CFU/mL				

(continued)

Table 5.3 (continued)

Polymeric antimicrobials	Tested microorganisms	Microbiological parameters/tests											References
QCS-*co*-MMA copolymers:	*Streptococcus mutans* ATCC 25175	Inhibition rate (%)											Song et al (2016)
Material 1 (physically prepared)	*C. albicans* ATCC 90028		*S.mutans*							*C.albicans*			
	Original inoculum:												
	$5–10 \times 10^5$ CFU / mL	Material 1	70–85							20–65			
Material 2 (chemically prepared)		Material 2	25–70							0			
A1-A8	S1*: S. aureus* ATCC 25923		DIZ (mm)							MIC and MBC/MFC (mg/mL)			Tuchilus et al. (2017)
	S2*: Sarcina lutea* ATCC 9341		S1	S2	S3	S4	S5	S6	S7	S1		S5	
										MIC	MBC	MIC	MFC
	S3*: E. coli* ATCC 25922	A1	14	14	0	0	11	13	13	1.25	2.5	2.5	5
	S4*: P. aeruginosa* ATCC 27853	A2	14	15	11	0	12	13	20	1.25	2.5	2.5	5
	S5*: C. albicans* ATCC 90028	A3	13	14	11	0	12	12	20	1.25	2.5	2.5	5
	S6*: C. glabrata* ATCC MYA2950	A4	13	13	0	0	12	12	20	1.25	2.5	2.5	5
	S7*: C. parapsilosis* ATCC 22019	A5	10	14	0	0	13	12	20	1.25	2.5	2.5	5
		A6	15	14	0	0	15	13	22	0.6	1.25	1.25	2.5
		A7	15	18	11	0	14	15	23	0.6	1.25	1.25	2.5
		A8	0	0	0	0	10	11	15	–	–	–	–

P-*g*-pAPTAC1	*S. aureus* ATCC 25923		Diameter of inhibition zone (mm)							Grigoras et al. (2013)
P-*g*-pAPTAC2		P-*g*-pAPTAC1	8.5 -10							
P-*g*-pAPTAC3		P-*g*-pAPTAC2	7 – 9							
		P-*g*-pAPTAC3	9 – 10							
QCG-1	S1: *S. aureus* ATCC 29213 (MSSA)	MIC (μg/mL) and MBC (μg/mL) of QCG derivatives against *Staphylococcus sp.*								Quelemes et al. (2017)
			QCG-1		QCG-2		QCG-3		Control	
QCG-2	S2: *S. aureus* ATCC 25923 (MSSA)		MIC	MBC	MIC	MBC	MIC	MBC	MIC	
QCG-3	S3: *S. aureus* MS52 (MSSA) (skin burn)	S1	1000	1000	250	250	125	125	< 0.25	
	S4: *S. aureus* ATCC 43300 (MRSA)	S2	1000	1000	125	125	125	125	< 0.25	
	S5: *S. aureus*-Col (MRSA)	S3	1000	1000	250	500	125	250	0.25	
	S6: *S. aureus* MR17 (MRSA) (surgical wound)	S4	500	1000	62.5	62.5	62.5	62.5	0.5	

(continued)

Table 5.3 (continued)

Polymeric antimicrobials	Tested microorganisms	Microbiological parameters/tests								References
	S7: *S. aureus* MR359 (MRSA) (surgical wound)	S5	1000	1000	125	125	62.5	125	0.5	
	S8: *S. aureus* MR0405 (MRSA) (nasal secretion)	S6	500	1000	62.5	125	31.25	62.5	0.5	
	S9: *S. epidermidis* ATCC 12228 (MSSE)	S7	1000	1000	125	250	125	125	0.5	
	S10: *S. epidermidis* MR 111 (MRSE) (blood culture)	S8	1000	>1000	125	250	62.5	125	0.5	
	S11: *S. epidermidis* 70D (MRSE) (blood culture)	S9	62.5	1000	31.25	250	31.25	250	0.5	
	Original inoculum:	S10	1000	1000	125	500	125	250	1.0	
	5×10^5 CFU/mL	S11	1000	1000	125	250	125	125	1.0	

PLA-N	*E. coli* ATCC 25922	Bacterial reduction R (%) in the presence of PLA derivatives				Logar et al. (2016)
PLA-Ag	*S. aureus* ATCC 6538					
PLA-SiQAC			c_{Ag} (mg/kg)	R (%)		
PLA-RV-Ag				*E.coli*	*S.aureus*	
PLA-RV-Ag-SiQAC	Original inoculum:	PLA-N	0	–	–	
		PLA-Ag	9	50	59	
	1–2 × 10^5 CFU / mL	PLA-SiQAC	0	68	78	
		PLA-RV-Ag	140	100	100	
		PLA-RV-Ag-SiQAC	53	100	100	
PLA film	*P. aeruginosa*	Bacterial reduction R (%) in the presence of PLA derivatives				Staneva et al. (2015)
B-PLA film	*E. coli*		R (%)			
			P. aeruginosa	*E. coli*		
		PLA film	7	19		
		B-PLA film	31	25		
Synthetic polymers						
Modified PAMAM dendrimers	*S. aureus*		MIC (µg/mL)	MBC (µg/mL)	DIZ (mm)	Maleki et al. (2017)
	B. subtilis					
(G4I)	*Klebsiella oxytoca*	*S. aureus*	52	64	31	
	E. coli	*B. subtilis*	50	67	28	
		Klebsiella oxytoca	55	71	26	
		E. coli	57	75	25	

(continued)

Table 5.3 (continued)

Polymeric antimicrobials	Tested microorganisms	Microbiological parameters/tests			References
PU foams	*E. coli* ATCC 25922	Antimicrobial efficiency (%) of PU foams toward bacteria strain			Udabe et al. (2017)
	S. aureus ATCC 29737		*E. coli*	*S.aureus*	
		PU foam 1	36	61	
		PU foam 2	38	1	
		PU foam 3	11	90	
		PU foam 4	2	4	
		PU foam 6	66	0	
QASs: 3a, 3b, 3c, 3d	*S. aureus*	MIC (μg/mL) for QASs			Liu et al. (2015)
PU=PU coating without QAS	*E. coli*		*S. aureus*	*E.coli*	
QAS-containing PU coatings:		3a	62.5	500	
PU(1)=compound 3b, 5 wt%		3b	31.25	250	
PU(2)= compound 3b, 9 wt%		3c	31.25	62.5	
PU(3)= compound 3b, 13 wt%		3d	15.63	62.5	
PU(4)= compound 3b, 16 wt%		Bacterial reduction R (%) in the presence of QAS-containing PU coatings			
PU(5)= compound 3a, 5 wt%			*S.aureus*	*E.coli*	
PU(6)= compound 3c, 5 wt%		PU	0	0	

PU(7)= compound 3d, 5 wt%		PU(1)		29.4		25.3		
		PU(2)		30.4		28.5		
		PU(3)		36.3		35.0		
		PU(4)		55		45.2		
		PU(5)		27.5		23.6		
		PU(6)		36.7		29.7		
		PU(7)		37.8		32.2		
P(SiO) m(SiOQAEp): P1 (M_w = 3150 g/mol)	*E. coli*	MBC (× 10^{-5} mol/L)						Cui et al. (2015)
P2 (M_w = 4670 g/mol)	*S. aureus*		P1	P2	P3	P4		
P3 (M_w = 7520 g/mol)	*Bacillus subtilis*	*E. coli*	1.4	0.5	0.65	0.1		
P4 (M_w = 13000 g/mol)		*S. aureus*	1.75	0.7	0.45	0.2		
		B. subtilis	2.7	1.5	0.25	0.3		
C_m-PSi-C_m (m= 8, 10, 12, 14, 16, 18)	*E. coli*	Zones of inhibition R (in mm) of 1.0 wt% C_m-PSi-C_m against microorganisms						Bao et al. (2017)
	S. aureus *Aspergillus flavus*		m= 8	m= 10	m= 12	m= 14	m= 16	m= 18
		E. coli	-	3.5	3	2	1.5	1.5
		S. aureus	2	4	3.5	3	2.5	2.5
		A. flavus	–	2	2.5	3	2.5	–

(continued)

Table 5.3 (continued)

Polymeric antimicrobials	Tested microorganisms	Microbiological parameters/tests					References
PDMS-*b*-QPDMAEMA40	*B. subtilis*	Antimicrobial activity of block copolymer films					Qin et al. (2015)
PDMS-*b*-QPDMAEMA60	*E. coli*		P-*b*-Q40	P-*b*-Q60	P-*b*-Q80	P-*b*-Q100	
PDMS-*b*-QPDMAEMA80		*B. subtilis*	+	+	+	+	
PDMS-*b*-QPDMAEMA100		*E. coli*	–	–	–	+	
Quaternized CBABC copolymers	*B. subtilis* ATCC 63501	Antimicrobial activity of the multi-block copolymer films					Zhou et al. (2015)
	E. coli ATCC 44752						
	Original inoculum:		*B. subtilis*		*E. coli*		
	10^5–10^6 CFU/mL	PDMS-QPM_{80}	+		+		
		PDMS-QPM_{80}-PF_{30}	+		+		
		PDMS-QPM_{80}-PF_{52}	+		+		
		PDMS-QPM_{80}-PF_{70}	–		–		

Quat-modified PE 1-9	*S. aureus*	Antibacterial activity of modified PE samples containing 5 wt-% quat (after leaching)				Rossetti et al. (2017)
		Quat-PE	*S. aureus*	*E. coli*	*P. aeruginosa*	
	E. coli	1	++	++	n.a.	
	P. aeruginosa	2	–	n.a.	n.a.	
		3	–	n.a.	n.a.	
		4	++	+/–	++	
		5	–	n.a.	–	
		6	++	++	++	
		7	+	+	++	
		9	+	–	++	
		Notes: The amount of quat 8 available was not sufficient for a thorough investigation; however, in preliminary experiments it was found to have good antibacterial activity against *S. aureus*; ++: most bacteria are dead; +: proportion of dead bacteria clearly increased compared to PE reference; +/–: different results with different samples; –: proportion of dead bacteria not increased compared to PE reference.				

Legend: CFU = colony forming units; CV = cell viability (%); DIZ = diameter of inhibition zone (mm); MIC = minimum inhibitory concentration; MBC = minimum bactericidal concentration; MFC = minimum fungicidal concentration;
Inhibitory index (%) = $(1 - D_a/D_b) \times 100$ (1)
where D_a is the diameter of the growth zone in the test plates and D_b is the diameter of the growth zone in the control plate
Zones of inhibition R (mm) = $(D_1 - D_2)/2$ (2)
where D_1 is diameter of transparent zone in mm, D_2 is diameter of 20 mm filter paper
$RC\text{-}QAS_{(C0)}$ = regenerated cellulose (RC) membrane including trimethoxysilylpropyl trimethyl ammonium chloride
$RC\text{-}QAS_{(C18)}$ = regenerated cellulose (RC) membrane including trimethoxysilylpropyl octadecyldimethyl ammonium chloride
CS; Cs; CTS; Ch = chitosan
NQC = N-trimethyl ammmonium chitosan chloride
Q1P3, Q1P1 and Q3P1 = hydrogels composed of N-quaternized chitosan (NQC) and poly(vinyl alcohol) (PVA) in different weight ratios (1:3), (1:1) and (3:1) chemically crosslinked by glutaraldehyde (GA) in different weight ratios (1.0 and 5.0%)
HACC/PVA/PEO hydrogel = hybrid hydrogel based on quaternary ammonium chitosan (HACC) combined with poly(vinylalcohol) (PVA) and poly(ethylene oxide) (PEO) obtained by γ-irradiated crosslilnking
QCh = *N,N,N*-trimethylchitosan iodide (quaternized chitosan)
Car = *k*-carrageenan

(continued)

Table 5.3 (continued)

CA/PHB = poly(3-hydroxybutyrate) PHB fibers containing caffeic acid CA
QCh/Car-coated PHB = PHB fibers coated with QCh/Car complex
QCh/Car-(CA/PHB) = CA/PHB fibers coated with QCh/Car polyelectrolyte complex
CA/QCh/Car-PHB = PHB fibers coated with QCh/Car complex containing CA
CTS–CTA = N-(2-hydroxy) propyl-3-trimethylammonium chitosan chloride
(CTS–CTA)–I_2 = complex of CTS–CTA with iodine (1: 1.33 molar ratio)
CSB = chitosan-bromoacetate
CSC = chitosan-chloroacetate
CSDC = chitosan-dichloroacetate
CSTC = chitosan-trichloroacetate
CSTF = chitosan-trifluoroacetate
TCTS = chitosan derivative containing 1,2,3- triazole without halogen
CTCTS, BTCTS = chitosan derivatives containing 1,2,3- triazole with halogen (Cl or Br)
BZK-Cs = chitosan grafted with 4-bromobutyl-benzalkonium bromide (BZK-Br)
TEA-Cs = chitosan grafted with 4-bromobutyl-triethylammonium bromide (TEA-Br)
PYA-Cs = chitosan grafted with 4-bromobutyl-pyridinium bromide (PYA-Br)
BTP-Cs = chitosan grafted with a mixture of 1.5 mmol each of BZK-Br, TEA-Br and PYA-Br (1:1:1 M_w ratio)
QAS-CS = *O*-quaternary ammonium salt-chitosans bearing *N*-methyl-N-R-N, N-bis(2-hydroxyethyl) ammonium bromides (R = –benzyl (chloride, BNQAS-CS), –dodecyl (C12QAS-CS), –tetradecyl (C14QAS-CS), –hexadecyl (C16QAS-CS), –octadecyl (C18QAS-CS))
Chitosan biopolymer dye = obtained by insertion of Reactive red x-3b (dye containing sulfonate groups) in chitosan quaternary ammonium salt (*N*-(2-hydroxyl)propyl-3-trimethyl ammonium chitosan chloride)
N-Q_xCS (x = 1–3) = *N*-quaternary ammonium chitosan derivatives from quaternization of CS with glycidyl trimethylammonium chloride GTMAC
Q_3B_yCS (y = 1–3) = *O*-sulfobetaine-N-Q_3CS = *N*-quaternary ammonium-*O*-sulfobetaine-chitosan (zwitterionic compounds)
OQCS = *O*-quaternary ammonium chitosan
OQCATUCS-1, OQCATUCS-3, OQCATUCS-5 = *O*-quaternary ammonium *N*-acyl thiourea chitosan derivatives with 77% degree of quaternization substitution DSQ and different degree of acyl thiourea DSAT substitution of chitosan (81%, 87% and 93%, respectively)
QCS-*co*-MMA copolymers = copolymers of chitosan quaternary ammonium salt (QCS) and methyl methacrylate (MMA)
Material 1 = composite prepared by directly adding of QCS to Denture Powder (containing polyMMA) followed by adding of then the Denture Water (containing MMA monomer and trace amount of the cross-linking agent, inhibitor and UV absorber)
Material 2 = composite prepared by adding varying amount of QCS to the Denture Water, allowing QCS and MMA monomer to engraft
A1-A8 = cationic amphiphilic dextran derivatives with a long alkyl group (dodecylamine, octadecylamine or di-*N*-dodecylamine type) attached to the reductive end of the polysaccharide chain and quaternary ammonium groups (*N*,*N*-dimethyl-*N*-octylamine, *N*,*N*-dimethyl-*N*-benzylamine or 1-methylimidazol type) attached as pendent groups in the presence of epichlorohydrin ECH to the main dextran backbone (with variable molecular mass: M_n (GPC) = 8000 or 4500 g/mol)
P-*g*-pAPTAC1–3 = grafted pullulans (P) with 22.53, 29.05, and 34.51 (wt.%) content of poly(3-acrylamidopropyl) trimethylammonium chloride pAPTAC

QCG1–3 = quaternized cashew gum with different substitution degree (0.42, 0.68 and 0.73) of 3-chloro-2-hydroxypropyl) trimethylammonium chloride CHPTAC
PLA-N = non-treated poly(lactic acid) fabrics
PLA-Ag = poly(lactic acid) fabrics physically bonded with Ag
PLA-SiQAC = poly(lactic acid) fabrics chemically bonded with 3-(trimethoxysilyl)-propyldimethyltetradecyl ammonium chloride
PLA-RV-Ag = poly(lactic acid) fabrics coated with crosslinked Si-matrix and Ag
PLA-RV-Ag-SiQAC = poly(lactic acid) fabrics coated with crosslinked Si-matrix, Ag and 3-(trimethoxysilyl)-propyldimethyltetradecyl ammonium chloride
PLA film = polylactic acid film
B-PLA film = cationic water soluble fluorescent B possessing amino groups (1-[(7-oxo-7H-benzo[de]anthracen-3-ylcarbamoyl)-methyl]-triethylammonium chloride) incorporated into thin polylactic acid film
Modified PAMAM dendrimers = modified poly(amidoamine) PAMAM dendrimers (generations G2 and G4) into quaternary ammonium salts using Cl, Br, I halogens groups (G2Cl, G2Br, G2I, G4Cl, G4Br, and G4I)
PU foams = poly(urethane) PU foams 1–9 with different quatermary ammonium content: PU foam 1 (5.4% quaternary ammonium content); PU foam 2 (7.2% quaternary ammonium content); PU foam 3 (containing poly(hexamethylene diisocyanate) pHDI and 16% quaternary ammonium content); PU foam 4 (containing toluene diisocyanate TDI); PU foam 6 (0% quaternary ammonium content)
3a-d = terpene-based carbamate group-containing QASs synthesized through the quaternization of terpene-based carbamate with alkyl bromides
QAS-containing PU coatings = polyurethane coatings containing quaternary ammonium salts; QASs incorporated into PU through crosslinking with terpene-based polyol and polyisocyanate (hydrophilically modified hexamethylene diisocyanate (HDI) tripolymer)
$P(SiO)_m$(SiOQAEp) = P1-P4 polysiloxane quaternary ammonium salt containing epoxy group obtained from polysiloxane containing tertiary amino group $P(SiO)_m$(SiOTA)n and epichlorohydrin by quaternization (where *m* is the length of hydrophobic (organosilicon group) chains, and *n* is the length of hydrophilic (quaternary ammonium group) chains) with *n* = 7.86–20.14, and *m*/*n* = 1.67–2.67
C_m-PSi-C_m = cationic gemini surfactants with polyether siloxane linked group and quaternary ammonium hydrophilic head group (C_m-PSi-C_m, m = 8, 10, 12, 14, 16, 18)
PDMS-b-QPDMAEMA = block copolymers based on poly(dimethylsiloxane) PDMS macro-chain transfer agent and poly(N,N-dimethylaminoethyl methacrylate (DMAEMA) quaternized with n-octyliodide
Quaternized CBABC copolymers = CBABC-type fluorosilicone multi-block copolymers (containing dithiocarbonate-terminated PDMS, PDMAEMA and PHFBMA) quaternized with n-octyliodide
PDMS-PM_{80} = BAB-type triblock copolymer PDMS-*b*-[PDMAEMA-*b*-P(DMAEMA-*co*-HEMA)]$_2$ containing poly(dimethylsiloxane) PDMS, poly(2-(dimethylamino)ethyl methacrylate) PDMAEMA and hydroxyethyl methacrylate HEMA
PDMS-QPM = quaternized copolymer PDMS-*b*-[PDMAEMA-*b*-P(DMAEMA-*co*-HEMA)]$_2$
PDMS-PM_{80}-PF_{30}, PDMS-PM_{80}-PF_{52} and PDMS-PM_{80}-PF_{70} = CBABC-type multi-block copolymers PDMS-*b*-[PDMAEMA-*b*-PHFBMA-*b*-P(HFBMA-*co*-HEMA)]$_2$ with three different contents of the poly(hexafluorobutyl methacrylate) PHFBMA blocks
PDMS-QPM-PF = quaternized multi-block copolymer PDMS-*b*-[PDMAEMA-*b*-PHFBMA-*b*-P(HFBMA-*co*-HEMA)]$_2$
Quat-modified PE 1–9 = composites of poly(ethylene) PE with mono- or bifunctional quaternary ammonium salts (benzyldimethylstearylammonium chloride (1); trimethylstearylammonium chloride (2); dimethyldistearylammonium chloride (3); methyltristearylammonium bromide (4); tetrastearylammonium bromide (5); 1,12-bis(dimethyloctylammonium)dodecane dibromide (6); 1,20-bis(dimethyloctylammonium)eicosane dibromide (7); 1,30-bis(dimethyloctylammonium)triacontane dibromide (8); bis(dimethyloctylammonium)PEG(400) ditosylate (9))

containing quaternary ammonium moieties. It could be observed that diameter of inhibition had values of 6–25 mm for quaternized compounds compared with their precursors characterized by lower or nonexistent antimicrobial activity. Also, for a constant concentration of potential biocidal polymer, diameter of inhibition zone increased in accordance with quaternization degree of polymers. In some cases, values of diameter of inhibition zone were lower than those recorded in presence of standard antibiotics (22–32 nm).

After a variable time of microorganisms incubation in presence of polymeric antimicrobials containing quaternary ammonium moieties, e.g. 4 h for hydrogels containing quaternized cellulose and native cellulose (Peng et al. 2016); 12 h for regenerated cellulose membranes including trimethoxysilylpropyl trimethyl ammonium chloride or trimethoxysilylpropyl octadecyldimethyl ammonium chloride (Meng et al. 2015) or 24 h for chitosan biopolymer dye (Tang et al. 2016), cell viability (%) was considerably reduced. This decrease in cell viability was inversely proportional to the alkyl chain dimension, substitution degree of ionizable groups in polymer chain and incubation time period. A similar behavior was observed in case of bacterial reduction. For example, for poly(urethane) coatings containing quaternary ammonium salts, this parameter had the highest value in case of compound with the longest hydrophobic alkyl chains of the whole series (Liu et al. 2015). In other case, if poly(lactic acid) fabrics coated with crosslinked Si-matrix contained 3-(trimethoxysilyl)-propyldimethyltetradecyl ammonium chloride and silver nanoparticles, the bacterial reduction represented a synergistic effect of quaternary ammonium groups and silver (Logar et al. 2016).

Because the quality and antiseptic properties of a substance are not assured only by a bacteriostatic effect, usually bactericidal activity is also examined (https://emerypharma.com/biology/minimum-inhibitory-concentration/). Literature reports that bacteriostatic activity is associated with a ratio of minimum bactericidal concentration to minimum inhibitory concentration greater than 4, although determination of this ratio is affected by numerous factors and technical problems. Regarding *in vitro* microbiological activity, it has been found that, at high concentrations, some antibacterial agents considered bacteriostatic are often bactericidal towards some susceptible organisms, while at low concentrations, other bactericidal drugs simply exhibit bacteriostatic effect. *In vitro* bacteriostatic or bactericidal data provide information about potential action of antimicrobial agents because only clinical results are relevant in treatment of bacterial infections. Thus, presumption that bactericidal agents are superior compared with bacteriostatic agents was invalidated by clinical results: meningitis, usually treated with bactericidal drugs, was effectively cured with bacteriostatic agents (Pankey and Sabath 2004).

In case of cationic amphiphilic dextran derivatives with a long alkyl group and quaternary ammonium moieties, minimum bactericidal concentration was two times lower than minimum inhibitory concentration; same results was recorded between minimum fungicidal concentration and minimum inhibitory concentration (Tuchilus et al. 2017). In the same time, values of minimum inhibitory concentration were insignificantly different from minimum bactericidal concentration values for the most quaternized cashew gums with different substitution degree of

(3-chloro-2-hydroxypropyl) trimethylammonium chloride (Quelemes et al. 2017), while for poly(amidoamine) dendrimers modified with halogenated quaternary ammonium salts, values of minimum bactericidal concentration were slightly higher than minimum inhibitory concentration values (Maleki et al. 2017). Study about antimicrobial activity of poly(siloxane) quaternary ammonium salts containing epoxy group revealed that microbiological parameters indirectly have been influenced by concentration of quaternary ammonium groups such that minimum bactericidal concentration values generally decreased with increase of molecular weight of polymers (Cui et al. 2015).

5.6 Factors That Influence Antimicrobial Activity

Beside already presented heterogeneous chemical composition of microorganisms responsible for hydrophilic, hydrophobic, lipophilic or amphiphilic character of microbial entities, there are other factors that influence antimicrobial activity of substances (Table 5.4). Higher is the *degree of substitution* of ionizable groups, more potent is the antimicrobial activity. Because of a large number of incorporated positive charges in modified polymers, *charge density* increases with increase in zeta potential values for macromolecules in solution. *Molecular weight of polymer* is another characteristic that could indirectly influence charge density. By increasing degree of substitution, molecular weight of polymer increase and more electrostatic interactions are established between biomembranes and antimicrobial substances (Wang et al. 2016; Quelemes et al. 2017). A higher antimicrobial activity was recorded with an increase in molecular weight, in case of poly(siloxane) quaternary ammonium salts containing epoxy group compared with their precursor (Cui et al. 2015).

Depending on *position of the biological active quaternary ammonium groups* in polymer structure (usually pendant, and rarely in side chain) and their *steric hindrance*, the *flexibility* and *conformation* of macromolecular chains may vary, influencing thus probability of interactions between cationic quaternary ammonium groups and anionic components of microorganisms (Bao et al. 2017). Flexible chains bearing quaternary ammonium groups have a facile access to components of microorganisms, while an extended conformation enhances antimicrobial effect (Grigoras et al. 2013). Also, *probability of electrostatic interactions* depends on *density/concentration of quaternary ammonium bearing grafts* on polymer backbone which is indirectly related to degree of substitution of ionizable groups (Grigoras et al. 2013). For example, in case of wastewater treatment industry, the number of grafts on polymeric backbone is important. Thus, flocculation properties of some quaternary ammonium type materials were enhanced with increase of grafting ratio, because cell wall of pathological *E. coli* was destroyed by electrostatic interactions established between modified starches and biomembranes (Huang et al. 2017).

Table 5.4 Physical, chemical and physicochemical factors that influence antimicrobial activity of compounds bearing quaternary ammonium moieties

Factors	References
Degree of substitution of macromolecules	Peng et al. (2016), Li et al. (2015)
Molecular weight of polymers	Wang et al. (2016), Quelemes et al. (2017), Cui et al. (2015)
Charge density of macromolecular chain	Chen et al. (2016), Tuchilus et al. (2017)
Zeta potential of antimicrobials	Chen et al. (2016), Tuchilus et al. (2017)
Position of biological active quaternary ammonium groups in polymer structure: pendant or lateral	Tuchilus et al. (2017)
Steric hindrance of quaternary ammonium groups	Bao et al. (2017)
Flexibility of macromolecular chains	Grigoras et al. (2013), Lv et al. (2018)
Conformation of polymer chains	Grigoras et al. (2013)
Pendant chain dimensions or length of alkyl chains between quaternary ammonium groups and polymer backbone	Tuchilus et al. (2017)
Hydrophilic–hydrophobic balance	Tuchilus et al. (2017)
Critical micelle concentration	Tuchilus et al. (2017)
Density of the grafts on polymer backbone	Grigoras et al. (2013)
Chemical structure of polymer; polyampholyte or polyzwitterionic character of antimicrobials	Chen et al. (2016), Tuchilus et al. (2017)
Electronegativity of different substituted groups	Tan et al. (2016), Li et al. (2016)
pH of medium and type of solvent used for formation of polymeric solutions	Meng et al. (2015)
Surface morphology of polymeric films	Rossetti et al. (2017)
Synergistic antimicrobial effect of different reactive groups	Li et al. (2015), Tang et al. (2015), Tan et al. (2016), Li et al. (2016), Oyervides-Munoz et al. (2017), Ignatova et al. (2016), Logar et al. (2016), Mohamed et al. (2015)

Taking into account *hydrophilic–hydrophobic balance*, some researchers designed antimicrobials with polyampholyte or polyzwitterion character. Presence of hydrophobic *alkyl chains with variable lengths* between hydrophilic quaternary ammonium groups and polymer backbone could be tuned from synthesis conditions; the length of alkyl chain being thus a critical parameter. In this way, chemical structure of polymer, namely ratio between hydrophobic moiety and hydrophilic part, has a decisive role on antimicrobial activity. If hydrophobicity is enhanced by a long alkyl chain, penetration of quaternary ammonium groups through a hydrophobic biological membrane, mainly composed from proteins and phospholipids, is

assured, and efficiency of antimicrobials with quaternary ammonium groups is improved (Meng et al. 2015; Bao et al. 2017). It seems that, usually, an octyl chain in structure of polymers is the best choice to design such polymeric antimicrobials containing quaternary ammonium moieties (Tuchilus et al. 2017; Qin et al. 2015). Since longer alkyl chains are more flexible, they can patch and mask the quaternary ammonium chains, so that electrostatic interactions will be limited and the antimicrobial potential of the substances will decrease (Lv et al. 2018).

Another issue concerns the correlation between self-aggregation state of quaternized macromolecules and their antimicrobial activity. Experiments revealed that some chemical agents were microbiologically active only in self-assembled form when minimum inhibitory concentration value was almost the same with that of critical micelle concentration; others were efficient at concentrations higher or lower than their critical micelle concentration. In addition, bacteriostatic or bactericidal character of a substance is dictated by relationship between minimum bactericidal concentration and minimum inhibitory concentration; for example, in case of quaternized dextrans, minumum inhibitory concentration recorded a value of 20 times greater than minimum bacterial concentration (Singh et al. 2008).

Electronegativity of different substituted groups with halogens in polymeric quaternary ammonium salts is a sensitive factor that influenced antifungal activities of halogenated derivatives of chitosan acetate (Tan et al. 2016) or of chitosan derivatives with halogeno-1,2,3-triazole (Li et al. 2016). It was observed that as electronegativity increased in the order trifluoromethyl > trichloromethyl > dichloromethyl > chloromethyl > bromomethyl, antifungal activity recorded same trend. Halogenated groups with stronger electronegativity have ability to draw more electrons from surrounding cationic amino groups of polymeric ammonium salts, and positive charge densities of cationic amino groups was consequently enhanced. Cationic amino groups possessing higher positive charge densities are more likely to interact with anionic components of fungal entities, such as mannan, glucan, proteins, and lipids, thereby blocking the proper transport of essential nutrients into cell.

pH of medium and type of solvent influence the polymer solubility, conformation of macromolecular chains and accessibility of cationic quaternary ammonium groups to anionic components of microorganisms. For example, chitosan, a biopolymer with intrinsec antimicrobial properties, become soluble in water if a small quantity of acid is added in solution such that glucosamine units are transformed into positively charged moieties. In addition, in order to increase solubility and antibacterial activity of this polysaccharide, introduction of substituting groups represented a feasible choice, especially in case of quaternary ammonium groups.

Compared with macromolecules in solutions, where antimicrobial activity depends especially on flexibility of macromolecular chains, in case of polymeric films containing quaternary ammonium moieties, surface morphology is related with position of biological active quaternary ammonium groups confined on film surface. On the other hand, *morphology, roughness and topography of surface* are in close relationship with antimicrobial activity. A surface with relatively smooth

morphology and lower surface roughness value presents an increased contact area favoring microorganism adhesion (Rossetti et al. 2017).

In order to increase antimicrobial performance of materials, researchers chemically modify macromolecules such that equipped them not just with quaternary ammonium moieties, but also with other types of reactive antimicrobial groups: acyl thiourea, triazole, halogen, benzalchonium or pyridinium (Li et al. 2015; Tang et al. 2015; Tan et al. 2016; Li et al. 2016; Oyervides-Munoz et al. 2017). When they made physical mixture, beside quaternized polymer bearing quaternary ammonium cations, they used molecules with antimicrobial properties like silver, *k*-carrageenan or caffeic acid (Ignatova et al. 2016; Logar et al. 2016). In some cases, the intrinsic antimicrobial properties of polymers like chitosan were combined with the properties of quaternary ammonium cations (Mohamed et al. 2015). In this way, dual antimicrobial materials have a *synergistic antimicrobial effect* against a broad spectrum of microorganisms.

5.7 Conclusions

Quaternary ammonium groups are versatile chemical entities with antimicrobial properties that could be introduced in various type of polymers in order to design more efficient biocides. Incorporation of quaternary ammonium groups or moieties can be accomplished by two pathways or approaches: chemical and physical. Each of them has advantages and disadvantages. Antimicrobial agents bonded on matrix by physical forces are released in a controlled manner into environment until their concentration decreases by leaching of antimicrobial system. In return, chemical bonded antimicrobial agents, accomplished by binding biocide moieties to polymer chains, form a permanent bio-barrier and act as a biological obstacle for microorganisms that come in contact with polymeric matrix. Although, from economical point of view, a permanent biocidal material is preferred over physically bonded material, the latter possess a higher specific surface area available for more contacts with pathogenic entities. Even if polymeric quaternary ammonium salts have various advantages like chemical stability, no volatility, and generally do not penetrate mucosa, biocompatibility and cytotoxicity tests are required in order to assess potential toxic effects before introducing them into clinical use.

Currently, there are numerous roughly qualitative studies in which antimicrobial effects are generally observed. For the progress of pharmaceutical industry and responsible use of resources, the need for simulation studies of molecular dynamics regarding interactions between quaternary ammonium compounds and model molecules, which have chemical structures similar to each component of the microorganism, should be considered. Also, it is desirable and should insist on design of materials with dual-antimicrobial functional groups to prevent resistance of the most aggressive microorganisms to antimicrobial substances.

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