Formation of contact active antimicrobial surfaces by covalent grafting of quaternary ammonium compounds

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A B S T R A C T
Different synthetic strategies for the formation of contact active antimicrobial materials utilizing covalent linkage of quaternary ammonium compounds (QACs) were reviewed. There is a demand to find methods that will prevent bacterial fouling without the release of antimicrobial agents, because biocides cause environmental pollution and promote the development of bacteria resistance mechanisms. The contact active antimicrobial surfaces may provide a useful tool for this purpose. The covalent surface grafting of QACs seems to be a feasible and promising approach for the formation of safe and effective antimicrobial materials that could be utilized for medical devices, food industry, water treatment systems and other applications. This manuscript reviews covalent attachment of QACs to form contact active antimicrobial materials based on glass, metals, synthetic and natural polymers. The review emphasizes the description of different synthetic methods that are used for the covalent linkage. Direct covalent linkage of QACs to the material surfaces, a linkage via auxiliary nanoparticles (NPs), or spacers, controlled radical polymerization techniques and a linkage to pre-activated surfaces are discussed. The physico-chemical properties and biological activity of the modified surfaces are also described. This review does not cover non-covalent grafting of QACs and incorporation of QACs into a bulk material.

1. Introduction

Control of microbial growth on material surfaces is a subject of high scientific and practical significance. Harmful microorganisms growing on surfaces of medical devices, food processing and water treatment systems, filters etc. leads to diseases, contamination, spoilage and other damages [1–3]. Microorganisms can also build biofilms on materials surfaces. Microbial cells embedded in biofilm are up to 1000 times more resistant in comparison with planktonic cells. Therefore, the biofilms present a persistent source of microbial contamination that can be hardly eliminated [4–6]. Numerous antimicrobial agents are currently used to prevent the undesired microorganisms’ growth. However, extensive usage of these agents may cause environmental pollution, public health damages and promote development of bacteria resistance mechanisms resulting in the formation of multidrug resistant species [7,8]. Thus, there is a current demand to find new treatments that will enable the control of microbial growth without massive release of antimicrobial agents to the environment [9,10]. In the recent decade, new strategies that aim to decrease the amount of used antimicrobial agents have been developed.

The first approach is superhydrophobic surfaces (contact angle value > 150°) that diminish bacterial adhesion, rather than killing them directly, and enable to reduce biofilm formation without the use of biocides [11–13]. The second approach is the contact active antimicrobial materials. In contact active materials an antimicrobial moiety is attached to a material surface and kills the bacteria without being released [14–18]. Unlike superhydrophobic surfaces that provide an elegant solution for bacterial adhesion prevention, contact active materials can be used in applications where the physical elimination of microorganisms is also necessary [19,20]. Since the antimicrobial agent is not released, the contact active materials do not contribute to the environmental pollution and also retain their effect after multiple usages. Therefore, this approach allows minimizing the amount of the active agent needed to prevent microbial growth [21,22]. Due to these environmental and operational advantages, contact active antimicrobial materials are of high applicative interest [23–27]. Numerous approaches for grafting of antimicrobial moieties on material surfaces, such as covalent linkage, sonication [28–30], hydrogen bonding [31,32], hydrophobic interactions, electrostatic interaction [33] and metal-coordination chemistry [34,35] were reported. Covalent linkage represents one of the most effective approaches for a formation of stable and reliable contact active antimicrobial materials [36,37]. The strong covalent linkage ensures that the bioactive compound will not migrate to the environment, which is extremely important for biomedical, water...
Quaternary ammonium compounds (QACs) have potent antimicrobial activity and were found to be effective against various bacteria, including multidrug resistant strains [40–43]. Although the working mechanism of the QACs is not fully understood, their antimicrobial effect is related to strong affinity and damaging interactions between the positively charged quaternary nitrogen of the QACs and the negatively charged head groups of acidic phospholipids in microbial membranes [44,45]. In addition, it was reported that the polarity and steric properties have significant effect on the antimicrobial potential of QACs [40]. Due to their high variability, low cost and outstanding antimicrobial activity, QACs have drawn much interest. Surface attachment of QACs towards formation of contact active antimicrobial materials is highly desired, since it can minimize toxicity and environmental damage of these compounds and diminish bacterial resistance promotion [42,46–48]. Jiao et al. recently published a review that describes potential toxicological and antimicrobial resistance of QACs including biomedical applications containing biomaterials that are based on randomized human clinical trials, the golden standard in contemporary medicinal science [43].

This manuscript reviews covalent grafting of QACs to form contact active antimicrobial surfaces based on various materials such as glass, metals, synthetic and natural polymers (Table 1). The review emphasizes description of synthetic methods that are used for the covalent linkage. Direct covalent linkage of QACs to the material surfaces, a linkage via auxiliary nanoparticles (NPs) or spacers, controlled radical polymerization techniques and a linkage to pre-activated surfaces are discussed. This review does not cover non-covalent grafting of QACs and incorporation of QACs into a bulk material.

2. Synthetic methods for covalent linkage of quaternary ammonium compounds (QACs)

There are two main synthetic approaches, “linking tail-active head” and “in situ quaternization of tertial amines”, that are utilized for covalent bonding of QACs on the materials surfaces.
2.1. Covalent linkage of QACs utilizing “linking tail-active head”

In order to keep the biological activity of QACs upon their grafting on the material surfaces, a “linking tail-active head” approach is often used. In this method, QAC contains linking moiety, tail, which is responsible for its covalent attachment, and the bioactive moiety, head, which remains unaltered and exposed to the bulk (Scheme 1).

2.1.1. Trialkyl silyl linking tail

One of the most utilized “linking tail-active head” strategies involves QACs that contain trialkoxysilyl moiety as a linking tail [49]. Alkoxysilanes are known as coupling agents that are capable of forming Si-O bonds with hydroxyl groups. This synthetic approach advances genericity since it is compatible with all OH group containing surfaces and robustness, as it results in a formation of a strong tridentate bond (Scheme 2).

2.1.1.1. Direct linkage of trialkoxysilyl-QACs to surfaces. Trialkoxysilyl-QAC grafting was first reported in 1972 by Isquith et al. [50]. 3-(trimethoxysilyl) propyldimethyloctadecyl ammonium chloride (Si-QAC) was added to the surfaces of various materials that then demonstrated antimicrobial activity. The surfaces remained active after repeated water rinses. In this work chemical characterizations, mechanism or stability studies were not described [48].

In 2007 Andersen and co-workers reported the covalent linkage of octadecyltrimethyl (3-trimethoxysilylpropyl) ammonium chloride (ODDMAC) on microbrillated cellulose (MFC) [51]. For this purpose, pre-hydrolyzed ODDMAC formed a reaction with MFC methanol/water suspension at room temperature. It was found that even low amounts of the immobilized QACs provide MFC with a substantial antibacterial activity. The amount of bacterial cells was observed on RC-QAC-C18 membranes, indicating strong antibacterial and antiadhesive properties. Upon grafting of QAC-C0, the surface energy of the modified membranes was significantly decreased. The researchers suggested that such decrease in surface energy provide the modified membranes with anti-adhesive properties that also contribute to the antimicrobial activity. The stability study shows that the modified membranes keep their antimicrobial properties at 25 and 50 °C in a wide range of pH (1.5, 3.5, 6.5 and 10.8). At 90 °C the antimicrobial properties were kept at pH 3.5–6.5 range. In the combination of extreme pH conditions (1.5 or 10.8) and 90 °C, the bacteria viability was partially restored [53].

Torkelson et al. attached dimethyloctadecyl [3-(trimethoxysilyl) propyl] ammonium chloride to silica sand. For this purpose, the sand was stirred in the coating solution (1.2% v/v QAC solution in ethanol at pH 3) for 24 h and then washed and dried in 100 °C oven overnight. The modified QAC-silica sand was used as a filling material for drinking water filter. 9 cm columns with QAC-grafted silica sand were tested for antimicrobial activity against E. Coli, MS2 Coliphage, Poliovirus type 3 and Adenovirus type 2 and compared to the columns that contained unmodified sand. The QAC-modified sand filtration demonstrated more effective filtering of bacteria and viruses than the unmodified filtration resulting in 1.7 log, 1.8 log 1.9 log and 0.36 log filtering of E. coli, MS2 coliphage, Poliovirus type 3 and Adenovirus type 2, respectively compared to 0.1-0.3 log removals of E. coli and MS2 by uncoated sand [52].

In 2013 Poverenov and co-workers reported covalent linkage of QACs onto glass, polyvinyl alcohol (PVA) and cellulose by one-step reaction [53]. The QACs density on the treated surfaces was determined by fluorescent test [54]. The modified materials demonstrated antimicrobial activity against Gram-positive (B. Cereus and A. Acinetobacter) and Gram-negative (E. Coli and P. Aeruginosa) bacteria. Stability studies confirmed the strong linkage of QACs at wide range of pH and temperatures. In order to release QACs from the surfaces, the combination of 90 °C and extreme pH values (1.5 and 10.1) was required.

Meng et al. performed direct covalent linkage of QAC onto regenerated cellulose (RC) membrane surfaces [55]. To the aqueous acidic (pH 4) solutions of trimethoxysilylpropyl trimethyl ammonium chloride (QAC-C0) or trimethoxysilylpropyl octadecyldimethyl ammonium chloride (QAC-C18) the wet RC membranes were added and stirred at room temperature for 4 h and then washed and dried. E. Coli and S. Aureus were used to evaluate the bacteriostatic and bactericidal properties of the modified membranes. The RC-QAC-C0 did not demonstrate antimicrobial properties. However, a significant reduction in the amount of bacterial cells was observed on RC-QAC-C18 membranes, indicating strong antibacterial and antiadhesive properties. Upon grafting of QAC-C18, the surface energy of the modified membranes was significantly decreased. The researchers suggested that such decrease in surface energy provide the modified membranes with anti-adhesive properties that also contribute to the antimicrobial activity. The stability study shows that the modified membranes keep their antimicrobial properties at 25 and 50 °C in a wide range of pH (1.5, 3.5, 6.5 and 10.8). At 90 °C the antimicrobial properties were kept at pH 3.5–6.5 range. In the combination of extreme pH conditions (1.5 or 10.8) and 90 °C, the bacteria viability was partially restored [53].
Poverenov and Zhang results agree and demonstrate that the tridentate Si–O linkage is stable and can be broken only at conditions that combine high temperature with extreme pH values. Such an observation is not surprising, as it is known that a combination of high temperature and highly acidic or basic conditions promotes hydrolysis of Si–O bonds [56].

Gottenbos et al. covalently attached 3-(trimethoxysilyl)-propyldimethylolactearyl ammonium chloride to silicone rubber. The reaction was carried out at 80 °C during 20 h using water as a solvent [57]. The antimicrobial activity of the modified and unmodified silicone rubber was tested on S. Aureus, S. Epidermidis, E. Coli and P. Aeruginosa. The viability of the adherent bacteria was investigated using a live/dead fluorescent stain and a confocal laser scanning microscopy. It was found that modified surfaces significantly reduce bacterial viability to less than 25% in comparison to the unmodified surfaces where 90% of the adherent bacteria were live [55].

Nikawa et al. grafted a 3-(trimethoxysilyl)-propyldimethyl-octadecyl ammonium chloride on titanium via Ti–O–Si coupling at 25 °C utilizing water as a solvent [58]. The microbial adherence and biofilm formation on the modified titanium surface were examined. It was found that the grafted QAC significantly reduced colonization of C. Albicans and S. Mutans by inhibition of initial adherence with hydrophobic octadecyl alkyl chain and killing of the adherent cells with the quaternary ammonium salt moiety. Such modified titanium surfaces were suggested by the authors to be used for inhibition of dental or denture plaque formation. Therefore, their cytotoxicity for the human gingival fibroblasts was examined and demonstrated appropriate safety.

2.1.1.2. Linkage of trialkoxysilyl-QACs to pre-activated surfaces. Numerous materials absence functional groups that are suitable for a direct linkage of QACs. For instance, polyethylene (PE), polypropylene (PP) and polystyrene (PS) are among the most used synthetic polymers and they have no inherent functional groups that can be used for covalent linkage. In such cases, surface pre-activation is required.

Poverenov's group reported utilizing air-ozonolysis as a readily available, safe and effective activation method for surface activation of PE and PS-based films. Ozone induced oxidation resulted in the formation of carboxylic groups that were then reduced to hydroxyl groups. Then, the long C18-alkyl chain containing, dimethylolactearyl [3-(trimethoxysilyl)] propylammonium chloride (TSAc), and the short C1-alkyl chain containing, trimethyl [3-(trimethoxysilyl)] propyl ammonium chloride (TSA), were grafted on the activated surfaces utilizing tridentate Si-O linkage (Scheme 3) [59].

The long C18-alkyl chain bearing PS-TSAc and PE-TSAc demonstrated dramatic antimicrobial effects against Cöli, S. enterica and B. Subtilis. The antimicrobial activity of PS-TSAc and PE-TSAc correlated with QACs densities on their surfaces, demonstrating 6.6 and 3.1 log CFU reduction for PS-TSAc and PE-TSAc, respectively. Interestingly, the polymer surfaces grafted with the short alkyl chain QAC, PS-TSAn and PE-TSAn, not demonstrate significant bacterial inhibition pointing on the importance of the alkyl chain length for antimicrobial activity of the grafted QACs [57].

In another work of Poverenov group, an electrochemical method was used to activate stainless steel. Electrodeposition of phenol p-diazonium salt was utilized as a source of OH moieties on the material surface [60]. This allowed a covalent linkage of 3-(trimethoxysilyl) propyl dimethylolactearyl ammonium chloride QAC. The entire reaction took place in water and resulted in effective antimicrobial stainless steel surfaces, which were shown to be active against Gram negative, and Gram positive bacteria species. Nearly total killing of E. Coli, B. cereus and multidrug resistant to P. aeruginosa was observed [58].

2.1.1.3. Linkage of trialkoxysilyl-QACs utilizing nanoparticles. Nanoparticles (NPs) were also utilized as auxiliary linkers for covalent linkage of trialkoxysilyl-QACs on the material surfaces. For instance, Rhee's group reported linkage of QACs to alginate NPs that were then grafted on cellulose textile fibers [61]. Sodium alginate (SA) was reacted with trimethoxysilylpropyl octadecylammonium chloride (TSA) and CaCl2 cross-linker was added. The formed NPs (SA–TSA) were covalently linked to hydroxyl groups of cotton fabrics. SEM studies revealed homogenous dispersion of SA–TSA NPs with 99 nm average size on the cotton surface. The cotton fabrics treated with 70 ppm NPs showed efficient antimicrobial activity. More than 99.99% reduction in viable cell counts of E. Coli and S. Aureus bacteria have been observed. The treated cotton fabrics were found to maintain their antimicrobial efficacy after 30 laundry cycles [59].

Jang’s group reported covalent binding of (trimethoxysilyl)-propyldimethylolactearyl ammonium chloride (QAC) to a surface of silica NPs. In addition, silica NPs were modified with octadecyltrimethoxy silane (Ods) that does not contain QAC in order to separate the antimicrobial effect from the hydrophobic effect. The silica/QAC and silica/Ods NPs were then grafted on glass surface (Fig. 1) [62]. Although silica/QAC and silica/Ods NPs had similar hydrophobicity, the QAC-Si demonstrated enhanced antimicrobial activity due to a presence of quaternary ammonium moiety. The average number of viable bacteria that adhered to the silica/Ods NPs grafted glass surface was 29.4%, 23.7%, and 16.3% for E. Coli, S. Aureus, and D. Geothermalis, respectively. However, the average number of viable bacteria that adhered to the he silica/QAC NPs grafted glass surface was 3.4%, 1.5%, and 0.4% for E. Coli, S. Aureus, and D. Geothermalis, respectively. Therefore, in addition to the bacteria repelling hydrophobicity of octadecyl substituent, the quaternary ammonium groups of QAC provide the modified surfaces with bactericidal capability [60].

Summarizing this subchapter, alkoxysilanes-based QACs can be easily linked to the surfaces of various OH–containing materials (glass, titanium, cellulose, silicone, PVA, etc.) or any other material (various

![Scheme 3](118x75 to 478x222). Modification of PS or PE (a) air-ozonolysis; (b) reduction with NaBH₄ or LiAlH₄ (c) C₁₈-TSAc or C₁-TSAc, methanol-water, 50 °C, 24 h [57]. The figure is reprinted with permission of Colloids and Surfaces Biointerfaces journal.
polymers, metals, etc.) after pre-treatment that provide the presence of OH groups on their surface. The strong tridentate Si–O covalent bonding advances stability of the prepared materials at wide range of pH and temperatures. The modified contact active materials demonstrated antibacterial properties with no leaching of the active agent.

2.1.2. Epoxy linking tail

Epoxy moiety could also be utilized for covalent linkage of QACs as was demonstrated by Bras and co-workers who prepared antimicrobial contact active microfibril cellulose (MFC) [63]. Cellulose fibers were activated by NaOH for 30 min at 65 °C to attain a high degree of substitution. Then 2,3-epoxypropyl trimethylammonium chloride (EPTMAC) was chemically grafted onto cellulose through nucleophilic addition of the alkali-activated hydroxyl groups of cellulose to the epoxy moiety of EPTMAC (Scheme 4). The reaction was performed at 65 °C for 5 h. It was found that in order to have an efficient QAC attachment, 5% NaOH solution and 4 mol of EPTMAC per mole of anhydroglucose unit should be used.

Conductometric titration was utilized to establish degree of substitution of the prepared QAC-MFC. An antimicrobial activity of the films prepared from the modified MFC was studied on B. subtilis, S. aureus and E. coli bacteria. It was found that degree of substitution (mol of QAC per mol of anhydroglucose units) have dramatic effect on the antimicrobial activity: at 0.04-0.08 mol/mol degree of substitution, the antibacterial effect was observed. When the degrees of substitution were increased to 0.15 mol/mol, the bacteriostatic effect was observed. No leaching of the antimicrobial agents was observed in this study [61].

Zhou’s group reported covalent linkage of 2,3-epoxypropyl trimethylammonium chloride to nanofibril cellulose (NFC) [64]. First, NaOH was added to provide sufficient activation of the cellulose surface. Afterwards, the QAC was attached through nucleophilic addition of the alkali-activated hydroxyl groups of cellulose to the epoxy moiety of QACs at 65 °C for 8 h. The trimethylammonium chloride content was measured by conductometric titration of chloride ions using AgNO3 and found to be 0.59–2.31 mmol/g. The modified NFC was used to prepare cellulose nanopapers that demonstrated 200 MPa tensile strength, high surface cationic charge density and high water absorbency (750 g/g). These QAC-modified films were suggested by authors to be used as ultrahigh water absorbents for dye removal from aqueous waste streams [62].

2.1.3. Benzophenone linking tail

An interesting approach that utilizes photo-cross-linkage of benzophenone moiety to C–H groups possessing surfaces, was reported by Locklin and co-workers [25]. Derivatives of QACs that contain benzophenone moiety as a linking tail were synthesized by a two-step synthesis (Scheme 5). 4-Methylbenzophenone was reacted with N-bromosuccinimide in the presence of 2,2′-azobis(2-methylpropionitrile) in cyclohexane to form 4-bromomethylbenzophenone that was then reacted with N,N-dimethylamino-4-bromomethylbenzophenone containing substrates such as plastics, textiles and alkylated glasses. The prepared BPAM was covalently grafted on various C–H bond-containing substrates under mild UV irradiation (254 nm, 6.5 mW/cm²) resulting in thin active layer < 50 nm. Due to the electron-withdrawing ammonium substituent on the benzophenone moiety, the surface grafting was rapid (less than 1 min) and required low UV intensity. The modified surfaces demonstrated thermal, chemical and mechanical stability. Biocidal activity of the prepared materials was examined on S. aureus and E. coli bacteria and showed 100% efficiency against both bacteria. In addition, it was shown that after the adhered bacteria were killed by the BPAM functionalized surface and the dead cells were washed or wiped away.

**Scheme 4.** Linkage of QAC to microfibril cellulose utilizing epoxy linking moiety [61]. The figure is reprinted with permission of Carbohydr. Polym.
using water, the antimicrobial activity of the coatings was restored [25].

2.2. In situ quaternization of tertiary amines to form QACs

An additional approach for a covalent grafting of QAC involves a one-step reaction of tertiary amines with alkyl halides that leads to in situ formation of quaternary ammonium salt on the material surfaces (Scheme 6). In order to perform such a reaction, tertiary amines should be first covalently linked to the materials surfaces.

2.2.1. Direct linkage of tertiary amines via trimethylsilyl group

Superhydrophilic surfaces that also possess antimicrobial properties were developed by Matyjaszewski and co-workers [65]. Poly-2-dimethylaminoethyl methacrylate (PDMAEMA) was prepared by atom transfer radical polymerization. Then, trimethylsilyl (TMOS) moieties were incorporated into the PDMAEMA chains through sequential quaternization of the tertiary amino groups in PDMAEMA with (3-iodo-propyl) trimethoxysilane (IPTMOS). The remaining tertiary amino groups were quaternized with bromoethane to form a quaternized polymer, PQDMAEMA [63]. The TMOS groups efficiently anchored the polymer onto glass substrates. The resulting superhydrophilic surfaces demonstrate high stability, anti-fogging characteristics, and easy cleaning characteristics. The linked quaternary ammonium groups containing PQDMAEMA matrix entirely diminished E. Coli bacteria [63].

A binary polymer composed of partially quaternized linear copolymer poly (DMAEMA-co-MMA) and ethylene glycol dimethacrylate (EGDMA) was formed and covalently grafted on glass slides by Ming's group utilizing three synthetic steps [66]. First, poly-2-dimethyl-aminoethyl methacrylate-co-methyl methacrylate (poly-DMAEMA-co-MMA) copolymer was synthesized upon free radical polymerization in 75/25 molar ratio of DMAEMA/MMA. Then, partial quaternization of poly (DMAEMA-co-MMA) with different amounts of 1-bromoundecane leading was performed and led to various QAC amounts in the copolymer. Finally, the polymer network was grafted on the glass surface. For this purpose, EGDMA and 2-hydroxy-4-(2-hydroxyethoxy)-2-methylpropionicophenone (HHMP) were dissolved in toluene to obtain homogeneous solution that was then spin-coated on a clean glass slide at 800 rpm for 15 s. The covalent linkage was done utilizing UV irradiation in ultraviolet cross-linker apparatus with HHMP as a photo-initiator. The modified surfaces that included 5 mol% (1.0 g copolymer and 0.063 g of 1-bromoundecane with respect to the DMAEMA units) of QACs in the copolymer presented best antimicrobial activity, based on contact killing mechanism (3.6 and 2.0 log reduction for E. Coli and S. Epidermidis, correspondingly). The modified surfaces that included 5 mol% were chosen following the anticipation that subsequent quaternization could alter the hydrophobic/hydrophilic balance in the copolymer. A zone-of-inhibition test verified lack of leaching of the antimicrobial agent. In addition to the antimicrobial properties, the grafted hydrophobic polymer provided modified surfaces with anti-fogging properties. Therefore, the authors suggested utilizing this method for manufacturing lenses, medical devices and transparent packaging materials [64].

2.2.2. Surface activation and linkage of tertiary amines to form QACs

Surfaces that do not possess appropriated functional groups were functionalized prior to the covalent grafting of tertiary amines. Denes's group reported in situ formation of QACs on the surface of stainless steel and cellulose-based filter paper using cold plasma technology [67]. Plasma-enhanced surface functionalization is a relatively cost-effective and environmentally friendly route to modify both inorganic and organic surfaces without altering the materials’ bulk properties. The stainless steel was activated by O2, after that, hexamethyldisiloxane (HMDSO) was added and then ethylenediamine was deposited on the silylated surface. All reactions were performed under cold plasma treatment. In the case of cellulose surface, the hydroxyl groups on the cellulose backbone were used for direct covalent attachment of ethylenediamine. Subsequent reaction of ethylenediamine with hexyl bromide and further methylation by methyl iodide resulted in a formation of tertiary amonium groups on stainless steel and cellulose surfaces. The bactericidal properties of modified materials were examined on S. Aureus and K. Pneumoniae. The modified stainless steel surfaces exhibited 99.9% and 98% reduction in S. Aureus and K. Pneumoniae, respectively. The bactericidal efficacy of the quaternized cellulose filter paper was found to be 96.8% for K. Pneumoniae and 98.7% for S. Aureus. The antimicrobial activity of the modified surfaces was also studied by time-dependent experiments. Bactericidal properties were shown in quaternized surfaces incubated with S. Aureus after 1 h. After 4 h no bacteria were detected. The quaternized surfaces incubated with K. Pneumoniae reached their optimal activity between 6 and 9 h [65].

2.2.3. In situ preparation of QAC utilizing tertiary amine nanoparticles

In situ formation of QAC on the polymer chains that possess tertiary amines were first reported at 1990’s [68,69]. Formation of QAC on the polymer chain presents bulk and not surface modification. Therefore, this topic is briefly covered in this review by describing several examples that intend consequent surface application of the modified polymers.
Domb et al. prepared antimicrobial quaternary ammonium polyethyleneimine (QA-PEI) nanoparticles via two synthetic methods using cross-linking with glutaraldehyde [70,71]. Polyethyleneimine (PEI) is a synthetic polymer that contains primary (25%), secondary (50%), and tertiary (25%) amino groups. The PEI linking was followed by reductive amination with octanal and further N-methylation with methyl iodide (Scheme 7) [69]. It was found that QA-PEI nanoparticles could be used as antibacterial additives for biomedical devices. The QA-PEI nanoparticles completely inhibited bacterial growth of S. Aureus and E. Coli at 80 μg/MI and 320 μg/MI, respectively.

In additional study of this group, the QA-PEI particles were embedded in restorative dental composite resins at 1% w/w [72]. Complete inhibition of S. Mutans growth was observed and this activity retained for at least 3 months. The incorporation of antimicrobial nanoparticles did not alter the original mechanical properties of the composite material. In another work, Domb et al. demonstrated the potential application of antimicrobial QAC nanomaterials for water disinfection [73]. Quaternary ammonium polyethyleneimine (QA-PEI) nanoparticles were synthesized using diiodopentane crosslinker at ethanol reflux for 24 h then, the formation of N-alkylation with iodocane was conducted. Thereafter, methylation of these particles with methyl iodide was carried out. The prepared nanoparticles were applied on polyethylene vinyl acetate or polyethylene methacrylic acid and dried. In the second stage, the tertiary amino groups of polyethyleneimine were converted into quaternary ammonium via one-step reaction with CH₃I at 60 °C for 12 h. Once this reaction occurred, the product (QPU) was precipitated using ethanol and dried. For membrane preparation, the DMSO solution of polyether sulfone (PES) and QPU was spin coated coupled with a phase inversion technique. The antibacterial activity against E. coli and S. aureus bacteria was investigated. For this purpose, two pieces of membranes were immersed in 2 mL of E. coli or S. aureus suspension at 10⁶ CFU mL⁻¹ and incubated in a shaking incubator at 37 °C for 2, 4, 6, and 8 h, respectively. The optical degree of the bacterial suspension was determined at the wavelength of 500 nm. The modified membranes demonstrated antibacterial activity against the E. coli and S. aureus. In addition, the composite membranes showed prolonged clotting times, decreased protein adsorption and suppressed platelet adhesion. These results demonstrate the potential of the prepared modified polyurethane membranes as readily available and easy-to-use materials for biological application such as blood adsorbent materials [72].

2.2.5. Controlled radical polymerization techniques for QAC deposition on surfaces

Polymers containing quaternary ammonium compounds could be prepared by polymerization of the corresponding monomers. However, the molecular weight and architecture of the prepared polymer should be controlled to obtain an effective antimicrobial activity. For this purpose, controlled radical polymerization techniques were developed. This method enables preparation of well-defined polymers with controlled molecular weight, composition, chain architecture, and site-specific functionality [44,75]. Jaeger et al. published a comprehensive review that describes the controlled free radical polymerization methods for synthesis of varied homo and block copolymers for fundamental investigations [76]. Different methods have been developed for controlled radical polymerization: (1) Stable free radical polymerization that involves thermal dissociation of dormant species (SFROP); (2) Atom transition metal-catalyzed atom transfer radical polymerization (ATRP) in which the transition metal complex Mn/L (SFRP); (2) Atom transition metal-catalyzed atom transfer radical polymerization (ATRP) in which the transition metal complex Mn/L performs homolytic cleavage of an alkyl halogen RX and generates the corresponding higher oxidation state metal halide complex Mn⁺¹X/L and an organic radical R that activates the dormant species and (3) Reversible addition fragmentation chain transfer (RAFT) [73,75]. The radical polymerization method allows versatility in monomer selection, relatively mild polymerization conditions and tolerance to various solvents and impurity. These advantages make this technique very promising for preparing quaternary ammonium compounds-based polymers from the perspective of industrial production and applications [43].

Matyjaszewski et al. prepared the first radically polymerize QAC-based antimicrobial material using the ATRP method. In this study, 2-(dimethylamino)ethyl methacrylate (DMAEMA) was directly grown on glass and paper surfaces via atom transfer radical polymerization. Subsequent quaternization of the amino groups of p(DMAEMA) generated a high concentration of QAC groups. The polymer-modified surfaces demonstrated substantial antimicrobial capacity against E. coli and B. subtilis [77].

RAFT technique allows the ability to synthesize well-defined polymers with various polar and nonpolar monomers under mild polymerization conditions. The most critical issue for successful RAFT polymerization is the selection of the suitable chain transfer agent. Commonly used chain transfer agents include dithioesters, xanthates, dithiocarbamates and trithiocarbonates [75]. Debashish et al. prepared antibacterial cellulose fibers utilizing RAFT polymerization. 2-(dimethylamino)ethyl methacrylate (DMAEMA) was polymerized on cellulose filter paper, afterwards the tertiary amino groups of the graft PDAEMA chains were quaternized with alkyl bromides of different chain lengths (C8-C16) to provide a large concentration of quaternary ammonium groups on the fiber surface. The antibacterial activity of the modified surfaces was found to correlate with the alkyl chain length and the degree of quaternization [78]. Singh et al. synthesized iodine...
containing quaternary amine methacrylate copolymers. Firstly, monomer was prepared by reaction of ethylene glycol dimethacrylate (EGDMA) with piperazine in methanol at 35 °C for 6 h. Then, quaternization of the synthesized monomer was performed with 1-iodoctane. Afterwards, the quaternized monomer was copolymerized with 2-hydroxyethyl methacrylate by radical polymerization using ammonium persulfate and N,N,N',N'-tетramethyl ethylenediamine as a redox initiator. The quaternized copolymers demonstrated antimicrobial activity against E. coli and S. aureus bacteria [79].

Huang et al. attached poly quaternary ammonium to polypropylene surface utilizing atom transfer radical polymerization (ATRP) using poly(2-dimethylamino)ethyl methacrylate) (PD-MAEMA) as a precursor. In the presence of ethyl bromide, the tertiary ammine groups in PDMAEMA were converted to QAC. The resulting surfaces grafted with high molecular weight polymers (Mn > 10,000 g/mol) demonstrated up to 100% killing efficiency against E. coli [52].

To summarize this chapter, various synthetic approaches should be used to achieve covalent linkage of QACs to a wide variety of material surfaces. The resulted materials demonstrate high antimicrobial potency without release of the biocide moiety to environment. These features categorize them as safe and environmentally friendly materials that can be used in numerous application such as filtering membranes, medical implants and devices, food contacting surfaces, water purification systems, etc.

3. Characterization of the contact active surfaces with covalently linked QAC

Various analytical tools could be used for qualitative and quantitative analyses of the modified surfaces after the covalent linkage of QACs is formed. In addition to the surface properties, the whole bulk materials characteristics are often examined after the modification.

3.1. Spectroscopic characterization

3.1.1. X-ray photoelectron spectroscopy

X-ray photoelectron spectroscopy (XPS) is widely used to characterize and describe contact active materials, including surfaces with covalently linked QACs. This method enables qualitative and quantitative characterization of materials surface. For instance, Poverenov et al. performed XPS studies for PVA-QAC, cellulose-QAC and glass-QAC [51]. Nitrogen peaks at 402.3–402.4 eV evidenced the surface grafting of QACs. The percent of the quaternized nitrogen (N +) was found to be 92.6%, 78.6% and 44% for PVA, cellulose and modified glass, respectively. This value correlated with the corresponding materials’ antimicrobial activity. In addition, XPS spectra shows that each trialkyl silyl moiety of QAC reacts with three OH groups on the material surface, confirming tridentate covalent binding. In another work, Poverenov et al. used XPS technique to follow three reaction steps required for covalent linkage of QAC to polyethylene and polystyrene surfaces: air-ozonolysis activation to form carboxylic groups, reduction to form hydroxyl groups and silylation to bind trialkyl silyl-QAC to the activated polymers surfaces [57].

Andersen et al. have utilized XPS method to monitor changes in chemical composition of the microfibrillated cellulose suspension upon reaction with octadecyl(dimethyl(3-trimethoxysilylpropyl) ammonium chloride (ODDMAC). The amounts of carbon, silicon and nitrogen in the surface increased with increasing the concentration of alkoxysilane groups [49]. Gottesbou et al. utilized XPS to confirm the presence of QAC on silicone rubber by quantitative monitoring of N and Cl peaks [55].

3.1.2. Fourier transform infrared spectroscopy

Fourier transform infrared spectroscopy (FTIR) technique utilizes infrared radiation to determine chemical bonds. Analysis of the FTIR spectra can reveal formation of new functional groups that takes place upon covalent linkage on the surface. In addition, it can be used to monitor the migration of functional groups to the polymer bulk. Attenuated total reflection (ATR) is a technique used in combination with FTIR and allows surface analysis of non-transparent materials. Majority of the studies that involve covalent binding of QACs utilize FTIR or ATR-FTIR for characterization of the modified products. Usually the spectra of the modified material is compared with an original one to get insight into the chemical bonding. For example, Izmaylov et al. confirmed linkage of imidazolium salts to the cotton fabric using FTIR by observing a characteristic peak of the imidazole ring at 1560–1567 cm⁻¹ [80]. FTIR spectra also helped Zanini et al. verify the attachment and the uniformity of the QAC on the polyurethane catheters [81]. Upon covalent linkage of QACs that possess an aliphatic chain and a siloxane-anchoring group, an increased C–H stretching (3000–2750 cm⁻¹) and Si–O stretching (1000–1100 cm⁻¹) are observed [57].

3.2. Determination of active site density

3.2.1. Conductometric titration

Titration methods for quantification of surface linked QACs are primarily based on counter anion exchange, for instance, reaction of quaternary ammonium chloride compounds with AgNO₃ salt. Pei et al. performed conductometric titration to cellulose nanofibers (Q-NFC) [62]. For this purpose, the Q-NFC sample was dispersed in Milli-Q water, and titrated with silver nitrate solution added as 0.2 mL in 60 s intervals. The amount of trimethylammonium groups in the surface was calculated based on the AgNO₃ volume used, assuming one chloride counter-ion per trimethylammonium group, and found to be 0.59–2.31 mmol/g. Saini et al. quantified quaternary ammonium groups on the pretreated nanofibber using conductometric titration of chloride ion with aqueous silver nitrate [61]. The degree of substitution (DS), 0.18, was calculated as follows: $DS = \text{ncellulose}/n\text{AgNO}_3$, where ncellulose is the number of anhydroglucose units in 1 g of cellulose and nAgNO₃ is moles of AgNO₃ in the volume used for titration.

3.2.2. Dye assay

To quantify surface linked QACs, colorimetric or chromogenic dye could be used. For instance, fluorescein dye selectively absorbs into the quaternary ammonium group and does not bind to tertiary, secondary or primary amines [51]. In order to determine the active site density, the QAC-modified surfaces are immersed in aqueous solution of fluorescein to bind it to QACs. The free dye is washed. Then, the competing quaternary ammonium agent, such as cetyltrimethylammonium chloride, is added in excess in order to desorb fluorescein from the surface linked QACs and to form a new cetyltrimethylammonium chloride-fluorescein complex in the solution. The absorption of this cetyltrimethylammonium chloride-fluorescein complex at 501 nm is measured to quantify the amount of fluorescein that was absorbed on the surface linked QACs [82].

3.3. Surface hydrophobicity

Water contact angle measurements provide a simple and rapid method that allows analyzing changes in surface hydrophilicity/hydrophobicity upon linkage of QACs [83]. Water contact angle data is used to calculate surface free energy. The Young–Dupré equation demonstrated the relationship between the surface free energy (E, mN/m), surface tension of water (γ, mN/m) and the contact angle (θ, °): $E = γ(1 + \cos θ)$ [53]. Surface free energy correlates with adhesion properties. Therefore, covalent linkage of long alkyl chain-containing QACs can significantly alter the surface free energy and affect the ability of bacteria to attach to such surface. For instance, Ment et al. found that the surface energy of the modified QAC-cellulose membranes is significantly lower than that of the unmodified cellulose (68.4 mN/m and 115.2 mN/m respectively). This reduction clarified the substantial decrease in bacteria adhesion to the modified surfaces that was observed in this study [53].
4. Microscopic characterisation

3.4.1. Atomic force microscope

Atomic force microscope (AFM) is used to determine morphology (topography and roughness) of the surface modified by covalent linkage of QACs. Pei et al. used AFM to study the morphology and size distribution of the surface quaternized cellulose nanofibers. They found that when the trimethylammonium chloride content was increased from 0.59 to 1.33 mmol/g, the width of the quaternized cellulose nanofibers decreased from 2.1 to 1.8 and the length from 2.0 to 1.3 mm [62]. Meng et al. prepared antibacterial cellulose membranes by direct covalent linkage of QAC. The AFM was utilized to measure the morphology and demonstrated an increasing in surface roughness in modified membranes to 69 nm, in comparison to pristine one that was found to be 53 nm [53]. Gao et al. covalently grafted dodecyl-alkylated quaternary ammonium to substrates containing C–H bonds such as plastics, fabrics, and alkyl-modified glass to form polymeric film. Upon modifications of QAC, AFM demonstrated decrease of roughness from 1.2 to 0.26 nm [25].

3.4.2. Scanning electron microscope

Scanning electron microscope (SEM) is widely used for surface analysis in general, and for morphological studies of QACs modified surfaces in particular. Cheng et al. prepared antimicrobial and antibacterial polymeric membranes by copolymerization of methyl acryloyloxgen ethyl trimethyl ammonium chloride (DMC) and poly (ethylene glycol) methyl ether methacrylate (PEGMA). SEM measurement indicated that the addition of DMC and PEGMA did not lead to dramatic changes in surface morphology and roughness. However, SEM images showed partial damage in the finger-shape structure of the composite membranes [84]. These observations correlated with the study of Wang et al. who prepared membrane composed of polyurethanes/polyethersulfone. SEM imaging demonstrated a clear finger-shape structure of the pristine membrane, that disappeared in the modified membranes. In addition, the pore sizes in modified membrane was changed [72]. Ahhua et al. utilize SEM for observation of quaternized cellulose nanofibers. When the trimethylammonium chloride content was 0.59 mmol/g, individual nanofibers with a width below 10 nm were observed together with nanofibril aggregates with a width of 10–30 nm. When the trimethylammonium chloride content was increased to 0.79 mmol/g, the nanofibrils became thinner and more homogeneous in lateral dimensions [62].

3.4.3. Fluorescence microscopy

Fluorescence microscopy is frequently used to follow the anti-microbial activity of the contact active surfaces. Hironobu et al. utilized fluorescence microscopy to follow antimicrobial activity of QAC-polymer matrix by observing green (live) cells and red (dead cells) of E. Coli. Once the surface QAC density exceeded to 3 × 10^{15} charge/cm², all the cells that were exposed to that surface were killed in short time [14]. Fadida et al. used fluorescence confocal microscopy to visualize GFP-labeled E. Coli contact with QAC-polyethylene and QAC-poly-styrene. The fluorescent bacteria were visualized using excitation wavelength of 488 nm and a BA 505–525 nm emission filter. After 30 min of contact with the modified surface, most bacteria lost their fluorescence, suggesting that the cells were killed [57].

3.5. Mechanical properties

Texture analyzer is often used to determine mechanical properties of materials. Upon covalent linkage of QAC to the surfaces of polymeric materials their elasticity, strength and Young’s modulus are usually measured. In most of the cases, such surface modifications do not affect the mechanical properties of the bulk material. However, in the case of thin materials surface modifications may have significant effect. For instance, Pei et al. prepared quaternized cellulose nanofibrils (Q-NFC), The nanopapers prepared from the Q-NFC have a Young modulus of ca. 10 GPa and tensile strength of ca. 200 MPa and the nanopaper prepared from unmodified NFC have a Young modulus of 13.2 GPa and a tensile strength of 214 MPa [64].

4. Future trends and possible applications

Covalent linkage of QAC to form contact active antimicrobial materials could provide a promising approach for reducing bacterial adhesion and proliferation. Such contact active antimicrobial materials could be used in water treatment systems, filters, aquatic flow systems, contact lenses, food industry, processing devices, medical instruments, ship hulls textile and clothes, everyday touched surfaces in the kitchen, computers, toys etc. Due to the stable covalent bond, the antimicrobial agents are not released into the environment even after multiple usages, thus, providing safety, ecological and economical advantages.

Although considerable progress has been made in this area and the experimental results are promising, many challenges remain. For the practical utilization, the fabrication process of QAC surfaces by industry should be easy, sustainable low-cost and consider the use of moderate condition (water as a solvent, room temperature, reasonable reaction time) and the price of the reagents involved. In this review, we have summarized different synthetic methods for the preparation of QAC surfaces. Although most of the described synthetic methods utilize water as the solvent and employ moderate conditions, more optimizations and improvements are needed. For instance, chemical pre-activation of unfunctionalized surfaces with oxidative agents (chronic, nitric or sulfuric acids, potassium permanganate or hydrogen peroxide) should be circumvented and there is a need to put more emphasis on a development of mild physical activation methods, such as cold plasma, ozone or electrochemical treatments. In addition, the synthetic process should be optimized to minimize the product purification and washing stages. The usage of organic solvents in product purification process should also be avoided.

Concerning possible future research, numerous directions have not yet been explored in this field. For instance, to the best of our knowledge, properties such as antifungal activity, odor absorbing, cleansing, antiseptics etc. of surfaces coated by QAC have not been investigated. We suppose that deeper study of this area would open new possibilities for utilizing QAC on coated surfaces to include anti-fungal and antiviral properties. In addition, a combination of QAC with additional active agents to receive poly-functional surfaces or synergistic effect is a promising research area of high potential. Combination of antimicrobial QACs with antiadhesive superhydrophobic or super-hydrophilic compounds could achieve a synergistic effect of the surface. Another research course could involve utilizing QAC surface linked to sensor materials that could enable signaling presence of bacteria.

5. Conclusions

The present manuscript reviews various synthetic approaches for covalent linkage of QACs to form contact active antimicrobial surfaces. QACs were successfully grafted on different synthetic and natural polymers, glass, and metal surfaces. All the treated surfaces show significant antibacterial activity without any leakage of the active agents.

From this survey, it appears that covalent linkage of antimicrobial QACs may establish a new general platform for developments of potent, safe and sustainable contact active antimicrobial materials, which can be easily utilized in broad range of industrial, medical and daily life applications. Moreover, QACs combined with additional active agents may result in new materials with multifaceted activity.

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