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Quaternary ammonium-based biomedical materials: State-of-the-art, toxicological aspects and antimicrobial resistance

[Yang Jiao](#),^{a,b,1} [Li-na Niu](#),^{a,1} [Sai Ma](#),^a [Jing Li](#),^c [Franklin R. Tay](#),^{d,*} and [Ji-hua Chen](#)^{a,*}

^aState Key Laboratory of Military Stomatology & National Clinical Research Center for Oral Diseases & Shaanxi Key Laboratory of Oral Diseases, Department of Prosthodontics, School of Stomatology, The Fourth Military Medical University, 710032, Xi'an, Shaanxi, China

^bDepartment of Stomatology, PLA Army General Hospital, 100700, Beijing, China

^cDepartment of Orthopaedic Oncology, Xijing Hospital Affiliated to the Fourth Military Medical University, 710032, Xi'an, Shaanxi, China

^dDepartment of Endodontics, The Dental College of Georgia, Augusta University, Augusta, GA, 30912, USA

Franklin R. Tay: ftay@augusta.edu; Ji-hua Chen: jhchen@fmmu.edu.cn

*Corresponding authors. jhchen@fmmu.edu.cn, ftay@augusta.edu

¹Equal contributors.

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Abstract

Microbial infections affect humans worldwide. Many quaternary ammonium compounds have been synthesized that are not only antibacterial, but also possess antifungal, antiviral and anti-matrix metalloproteinase capabilities. Incorporation of quaternary ammonium moieties into polymers represents one of the most promising strategies for preparation of antimicrobial biomaterials. Various polymerization techniques have been employed to prepare antimicrobial surfaces with quaternary ammonium functionalities; in particular, syntheses involving controlled radical polymerization techniques enable precise control over macromolecular structure, order and functionality. Although recent publications report exciting advances in the biomedical field, some of these technological developments have also been accompanied by potential toxicological and antimicrobial resistance challenges. Recent evidenced-based data on the biomedical applications of antimicrobial quaternary ammonium-containing biomaterials

that are based on randomized human clinical trials, the golden standard in contemporary medicinal science, are included in the present review. This should help increase visibility, stimulate debates and spur conversations within a wider scientific community on the implications and plausibility for future developments of quaternary ammonium-based antimicrobial biomaterials.

Keywords: Quaternary ammonium compounds, Antimicrobial surfaces, Toxicological aspects, Antimicrobial resistance

Nomenclature

AAM Acrylamide
AME Antimicrobial enzyme
AMP Antimicrobial peptide
ATRP Atom transfer radical polymerization
BPEA 2-(2-Bromopropionate)-ethyl acrylate
CCR5 C—C chemokine receptor type 5
CRP Controlled radical polymerization
CTA Chain transfer agent
CuAAC Cu-catalyzed azide–alkyne cycloaddition
DADMAC Diallyl-dimethylammonium chloride
DBCO Dibenzocyclooctynes
DCAMA Dipicolyl aminoethyl methacrylate
DDMAI 2-Dimethyl-2-dodecyl-1-methacryl- oxyethyl ammonium iodine
DEPN *N-tert-butyl-N*-[1-diethylphosphono- (2,2-dimethylpropyl)]
DMADDM Dimethylaminododecyl methacrylate
DMAEDM Dimethylammoniummethyl dimethacrylate
DMAEMA 2-Dimethylamino ethyl methacrylate
DMAE-CB Methacryloxyethyl cetyl ammonium chloride
DMAHDM Dimethylaminohexadecyl methacrylate
DODAB Dioctadecyldimethyl-ammonium bromide
EA Ethyl acrylate
EDAX Energy dispersive X-ray
EPSiQA Epoxy silicone quaternary ammonium salt
EPS Exopolysaccharides
FAc Fluorinated acrylate-heptadecafluorodecyl acrylate
FE-SEM Field emission scanning electron microscopy
FRP Free radical polymerization
GIC Glass ionomer cement
gtf Glucosyltransferase
GTAC Glycidyltrialkylammoniumchloride
GTMAC Glycidyltrimethylammonium chloride
GTEACl Glycidyltriehtylammonium chloride
HACC Hydroxypropyltrimethyl ammonium chloride chitosan
HDPC Human dental pulp cell
HEMA 2-Hydroxyethyl methacrylate
HGF Human gingival fibroblast
HSV Herpes simplex virus
IC₅₀ Half maximal inhibitory concentration
IDMA Ionic dimethacrylate

IMQ *N,N*-bis[2-(3-(Methacryloyloxy) propanamido)-ethyl]-*N*-methylhexa- decyl ammonium bromide
LCST Lower critical solution temperature
LC₅₀ Lethal concentration that kills 50% of a sample
MAE-DB 2-Methacryloxyethyl dodecyl methyl ammonium bromide
MBC Minimum bactericidal concentration
MDPB 12-Methacryloyloxy dodecyl pyridinium bromide
MFC Minimum fungicidal concentrations
MIC Minimum inhibitory concentration
MMA Methyl methacrylate
MMP Matrix metalloproteinase
Monomer II *N*-Benzyl-11-(methacryloyloxy)-*N,N*- dimethylundecan-1-aminium fluoride
MRSA Methicillin-resistant *Staphylococcus aureus*
MRSE Methicillin-resistant *Staphylococcus epidermidis*
MTT 3-(4,5-Dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide
MUPB Methacryloyloxyundecylpyridinium bromide
NACP Nanoparticles of amorphous calcium phosphate
NIPAAm *N*-Isopropylacrylamide
NM Not mentioned
NMR Nuclear magnetic resonance
NMP Nitroxide-mediated polymerization
NO Nitric oxide
NP Nanoparticle
Nrf2 Nuclear factor E2-related factor 2
P(AA-co-Ada) Poly(acrylic acid-co-1-adamantan- 1-ylmethyl acrylate)
PAMAM Poly amidoamine
PAH Poly(allylamine hydrochloride)
PB Poly(1-butene)
PCL-PJDMA Poly caprolactone-poly quaternary ammonium salt
pDADMAC-PU Poly diallyl-dimethylammonium chloride-polyurethane
PDMS Poly(dimethylsiloxane)
PEG Polyethylene glycol
PMMA Poly(methyl methacrylate)
PTEA 2-Phenyl-2-(2,2,6,6-tetramethyl- piperidin-1-yloxy)ethyl acrylate
PU Polyurethane
QA Quaternary ammonium
QAB Quaternary ammonium bromide
QAC Quaternary ammonium compound
QADM Quaternary ammonium dimethacrylate
QADMA Quaternary amine dimethacrylate
QADMAI-12 *N,N*-bis[2-(3-(Methacryloyloxy)- propanamido)ethyl]-*N*-methyl dodecyl ammonium iodide
QADMAI-16 *N,N*-bis[2-(3-(Methacryloyloxy)- propanamido)ethyl]-*N*-methylhexadecyl-ammonium iodide
QADMAI-18 *N,N*-bis[2-(3-(methacryloyloxy)- propanamido)ethyl]-*N*-methyloctadecyl ammonium iodide
QAES Quaternary ammonium epoxy silicate
QAMA Quaternized ethylene glycol dimethacrylate piperazine octyl ammonium iodide
QASM Quaternary ammonium salt containing monomers
QCS Quaternary ammonium chitosan derivative
QPEI Quaternary ammonium polyethylenimine
QPGMA Quaternized poly glycidyl methacrylate

RAFT Reversible addition fragmentation chain transfer
ROS Reactive oxygen species
RCT Randomized controlled trial
ROP Ring opening polymerization
SEM Scanning electron microscopy
SIP Surface-initiated polymerization
SNP Single nucleotide polymorphism
SPAAC Strain-promoted cycloaddition of alkynes and azide
SPH 5,5-Dimethyl-3-(3'-triethoxysilyl-propyl)hydantoin
SPMA 3-sulfopropylmethacrylate
SPODA 3-(Trimethoxysilylpropyl) octadecyl dimethyl ammonium chloride
TBAEMA 2-*tert*-Butylamino ethyl methacrylate
TEM Transmission electron microscopy
Ti Titanium
TGA Thermogravimetric analysis
TMSPMA Poly(3-(trimethoxysilyl)propyl methacrylate)
TPGDA Tripropylene glycol diacrylate
XPS X-ray photoelectron spectroscopy
XRD X-ray diffraction
Zn Zinc
2AE [2-(Acryloyloxy)ethyl]trimethyl- ammonium chloride
4VP 4-Vinylpyridine
8QA 8-Quinolinylnyl acrylate

1. Introduction

Microbial infection is a major challenge to human health worldwide. Pathogenic microorganisms, including bacteria, viruses and fungi, are of special concern in hospitals and other health care settings, and adversely affect the optimal functioning of medical devices, drugs, surgical equipment, dental restorations and bone cements [1]. According to a systematic analysis, infectious diseases result in 9.2 million deaths in 2013 alone (about 17% of all deaths), most of which are associated with biofilm formation [2], [3]. The discovery that microorganisms cause infectious diseases resulted in development of antibiotics, disinfectants and antiseptics against those microbial pathogens [4], [5]. However, the widespread and injudicious use of antibiotics and disinfectants has resulted in the emergence of antibiotic-resistant bacterial strains that are becoming a serious threat to human health [6]. There has been a constant race between researchers developing new antimicrobial agents and bacteria acquiring resistance to those agents. According to the U.S. Center for Disease Control and Prevention, more than two million people are infected with antibiotic-resistant bacteria and at least 23,000 patients die annually from those infections [7]. The World Health Organization 2014 report on global surveillance of antimicrobial resistance surmised that the world is heading towards a post-antibiotic era in which what used to be common, treatable infections are rapidly becoming life-threatening diseases. The organization has appealed to researchers worldwide to prioritize their efforts toward concerted efforts in combating the spread of antimicrobial-resistant microorganisms [8]. Over the past decade, new strains of infectious pathogens, such as severe acute respiratory syndrome, antibiotic-resistant tuberculosis, avian influenza A (e.g. H5N1, H7N9), the Ebola and Zika viruses have emerged. These new life-threatening pathogens have the tendency to spread globally instead of being confined to their niches of origin. Pragmatically, there is increasing need for exploring more efficient, broad-spectrum and long-lasting antimicrobial agents for biomedical applications.

To circumvent the uneven release kinetics and reservoir exhaustion issues of release-based antimicrobial biomaterials, contact-killing surfaces incorporating immobilized bactericides have been developed in which monomeric antimicrobial agents are covalently attached to the polymer backbone [9]. Compared with release-based biomaterials, the contact-killing approach have distinct advantages, in that they: (1) improve and prolong antimicrobial activities; (2) possess non-toxic and non-irritant properties without affecting the interaction with host tissues or modifying the host immune responses; (3); exert no adverse effects on the physical and mechanical properties of the loading materials; (4) are different from antibiotics in that their mode of action involves physically puncturing and destroying bacterial cell walls and membranes as well as viral envelopes [10]; and (5) are unlikely to develop antibiotic resistance [11]. The most commonly used antimicrobial compounds include quaternary ammonium compounds (QACs), chitosan, silver nanoparticles (AgNPs), antimicrobial peptides (AMPs) and antimicrobial enzymes (AMEs) (Table 1).

Table 1

Major antibacterial materials and their mechanisms of action.

Material type	Representative compounds	Mechanisms of action	Refs.
Antibiotics	Aminoglycosides (e.g. gentamicin, tobramycin)	Bind to the bacterial 30S ribosomal subunit and inhibit protein synthesis	[12]
	Glycopeptides (e.g. vancomycin)	Bind to amino acids and disrupt cell wall peptidoglycan synthesis	
	Penicillins (e.g. ampicillin)	Inhibit related enzymes and disrupt cell wall peptidoglycan synthesis	
	Quinolones (e.g. ciprofloxacin, norfloxacin)	Inhibit DNA replication and transcription, targeting DNA topoisomerases II and IV	
	Rifamycins (e.g. rifampin)	Bind to RNA polymerase and inhibit transcription	
	Tetracyclines (e.g. minocycline, tetracycline)	Inhibit protein synthesis	
Antimicrobial enzymes (AMEs)	Lysozyme	Catalyze glycosidic bond hydrolysis in bacterial cell wall peptidoglycans	[13]
	Acylase	Quorum-quenching	
Antimicrobial peptides (AMPs)	Natural AMPs (e.g. human β -defensin 1–3, magainin and nisin)	Transmembrane pore formation, intracellular targeting and metabolic inhibition mechanisms (inhibition of microbial functional proteins, DNA and RNA synthesis)	[14], [15]
	Synthetic AMPs (e.g. β -17, human neutrophil peptides 1 and 2, histatins 5 and 8)		
Cationic compounds	Chitosan	Interaction between positively charged chitosan molecules and negatively charged bacterial cell membranes leads to disruption of cell membrane	[16]
	Chlorhexidine	Bind to negatively charged bacterial walls and disrupt cell walls	
	Poly(ϵ -lysine)	Electrostatic adsorption onto bacterial cell membranes and stripping of the outer membrane lead to cell death	
	Quaternary ammonium compounds (QACs)	Disruption of bacterial enzymes and cell membranes by positively charged polymers	
Metal and metal oxides	AgNPs	Induce oxidative stresses, deactivate bacterial enzymes by binding to thiol groups and affect the function and permeability of the cell membranes	[17], [18]

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The use of QACs as antiseptics and disinfectants dated back to the 1930s [23]. The QACs employed in that era provided the first line of defense against pathogenic bacteria [24]. Structurally, QACs are composed of nitrogen (N^+)-containing compounds in which the N atom is attached to four different groups by covalent bonds. The representative formula is $N^+R_1R_2R_3R_4X^-$, where R may be a hydrogen atom, a plain alkyl group or an alkyl group substituted with other functionalities, and X represents an

anion, which is most often a halide anion. Most QAC salts are composed predominantly of chloride or bromide salts, while iodide salts tend to exhibit decreased solubility. Synthetic polymers with quaternary ammonium (QA) functionalities are produced using two general approaches: (1) quaternization of reactive precursor polymers (post-polymerization). This method generally generates products with variable degrees of cationization as a result of the unpredictable impact of steric hindrance from neighboring groups, and (2) direct copolymerization of monomers containing QA functional groups, coined as QA monomers, within the polymer network. These methods generate polymers with 100% functionality but with difficult molecular characterization ([Fig. 1](#)).

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[Fig. 1](#)

Schematic illustration of the use of quaternary ammonium compounds in antimicrobial biomedical materials.

There are already excellent reviews that summarize the synthesis of antimicrobial polymers and their chemical structures [\[16\]](#), [\[25\]](#), [\[26\]](#), [\[27\]](#), [\[28\]](#). Hence, the present review is targeted toward providing an overview of the state-of-the-art of QACs and their antimicrobial applications in the biomedical field ([Fig. 2](#)), and describing the currently-accepted mechanisms on how antibacterial, antifungal, antiviral and the recently discovered anti-matrix metalloproteinase (MMP) activities of QACs are achieved. The use of controlled radical polymerization (CRP) techniques, in particular, has provided a major thrust in the synthesis of novel QA-based antimicrobial surfaces. Because of increasing concerns over the biocompatibility of QACs, a critique on the toxicological aspects of QACs is also included. Similar to antimicrobial peptides, QACs are potential candidates for combating antibiotic resistance. Thus, the potential impact of QACs on the emergence of antibiotic resistance is also discussed. Apart from reviewing bench-top work and animal studies, recent evidenced-based data on the biomedical applications of antimicrobial QA-containing copolymers based on randomized human clinical trials will also be highlighted in the present review.

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[Fig. 2](#)

Chemical structures of representative quaternary ammonium compounds in antimicrobial biomedical materials.

2. Bioactive functions of QACs

2.1. Antibacterial activities

Quaternary ammonium compounds are cationic surfactants and antimicrobials with a broad spectrum of activities [29] (Table 2). Although QACs are lethal to a wide variety of organisms, including vegetative cells of gram-positive and gram-negative bacteria, fungi, parasites (e.g. *Leishmania major*; *Plasmodia falciparum*), and lipophilic (enveloped) viruses [30], [31], [32], they are generally not considered sporicidal (e.g. *Bacillus subtilis*, *Clostridium sporogenes*), tuberculocidal (e.g. *Mycobacterium tuberculosis*, *Mycobacterium bovis*) or virucidal against hydrophilic (non-enveloped) viruses (e.g. *Coxsackievirus*, *Rhinovirus*) [27]. Although the exact antimicrobial mechanism of QACs has not been fully elucidated, it is generally believed that the predominant mode of action is disruption of the cell membrane [33]. The antimicrobial effect of QACs has been attributed to a multitude of factors [16]. The molecular weight of QACs has a profound impact on the efficacy of many QAC-based antimicrobial systems [34], [35]. The length of the *N*-alkyl chain affects the antimicrobial activities of QACs [36], [37], [38]. For different bacteria and fungi, the optimum chain length of QACs is different (14 carbons for gram-positive bacteria, 16 carbons for gram-negative bacteria and 12 carbons for yeast and filamentous fungi) [39], [40]. Counter anion has a profound effect on the efficiency and selectivity toward different microbes due to discrepancies in polymer morphology, binding affinity toward quaternary compounds and the solubility of polycations in water, resulting in variable degrees of antimicrobial performance [41], [42], [43], [44]. Some researchers found that the antimicrobial activity of QA dendrimers is dependent on the counter anion and biocides with bromide anions are more potent than those with chloride anions [35]. Conversely, other studies reported that counter anion species have no effect on antibacterial activities [45], [46]. In addition, molecular charge density also affects the antimicrobial actions of QACs [47], [48], [49]. For example, a threshold of immobilized surface QA groups is required to cause significant reduction in viable bacteria [49].

Table 2

Antimicrobial activities of representative QACs against various pathogenic microorganisms and their cytocompatibility.

Microorganism	QACs	Antimicrobial activity	Related niche	Cytotoxicity
<i>A. israelii</i>	MDPB	MIC 12.5 µg/mL [50] MBC 25.0 µg/mL [50]	A/C/E	0–40 µg/mL for 48 h. No cytotoxicity to HDPCs [51] 0–50 µg/mL for 3d. No cytotoxicity to mouse odontoblast-like MDPC-23 cells [52] IC ₅₀ 62.5–75 µM to mouse osteoblast-like MC3T3-E1 cells [53]
<i>A. gerencseriae</i>	MDPB	MIC 3.13 µg/mL [50] MBC 6.25 µg/mL [50]	A/E	
<i>A. naeslundii</i>	MDPB	MIC 3.13– 25.0 µg/mL [50] MBC 6.25– 50.0 µg/mL [50]	A/C/E	
	QAMP	MIC 20 µg/mL [54] , [55] MBC 20 µg/mL [54] , [55]		Not assessed
<i>A. odontolyticus</i>	MDPB	MIC 6.25 µg/mL [50] MBC 12.5 µg/mL [50]	A/C/E	
<i>A. viscosus</i>	DMADDM	MIC 4.9 µg/mL [56] MBC 9.8 µg/mL [56]	A/C/E	LC ₅₀ 20–40 µg/mL to HGFs [56]

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Related niche. A: apical periodontitis; B: bone infections; C: caries; E: endodontic infections; W: wound infections.

2.2. Antifungal activities

Fungi have a single membrane surrounded by a thick cell wall composed of glucan and chitin, similar to the cell envelope of gram-positive bacteria [71]. Previous studies attributed the antifungal mechanism of QACs to electrostatic interaction with the fungi cell membrane that results in cell lysis [72], [73], [74]. More recent work on QACs reported a different antifungal mechanism. In-situ hybridization with fluorescent oligonucleotide probes shows that fungi express hyphae on untreated prostheses but not on QAC-coated prostheses. This observation suggests that the antifungal activities of QACs may involve impediment of hyphae formation [75]. The micelle-forming cationic detergent cetyltrimethylammonium bromide (CTAB) does not cause disruption of the fungal cell membrane [76]. Instead, its antifungal activity may be associated with the reversal of cell surface charges from negative to positive, as determined from measurements of the cell electrophoretic mobility. The antifungal activities of QACs are largely dependent on molecular structures [77]. To cause fungus death, the cationic QA moiety has to be adsorbed onto the cell, alter the inherent charge of the cell wall and penetrate the latter to reach the fungal cell membrane. The antifungal activity of the bilayer-forming, cationic synthetic lipid dioctadecyldimethyl-ammonium bromide (DODAB) has been reported to be not as strong as CTAB. This phenomenon may be attributed to cell aggregation as a function of cell concentration [78]. Fungal cells that are inside cell aggregates cannot be reached by DODAB and those cells remain viable. Conversely, the aggregated cells are apparently defenseless in the presence of CTAB. These results suggest that CTAB molecules penetrate *Candida albicans* aggregates more effectively to exert their antimicrobial activity. In addition, the rigid gel state of the DODAB molecule may hamper its penetration into the fungal cell wall and cytoplasmic membrane [79].

The mode of action of gemini QACs appears to involve lysis of the cell membrane and organelles without fungal cell wall destruction or protein leakage. Gemini QACs containing two pyridinium residues [3,3'-(2,7-dioxaoctane)bis(1-decylpyridinium bromide)] per molecule. These residues possess fungicidal activity against *Saccharomyces cerevisiae* by causing respiration inhibition and cytoplasmic leakage of adenosine triphosphate, magnesium, and potassium ions [80]. Gemini QACs exhibit more effective antifungal activity in comparison with mono-QACs *N*-cetylpyridinium chloride [81]. In addition, the activity of the gemini surfactant against *S. cerevisiae* and *C. albicans* results in elevated levels of reactive oxygen species (ROS) under aerobic conditions. The potent antifungal activity of D-glucosamine QA derivatives against *Coriolus versicolor* and *Poria placenta* may be explained by their capacity to recognize different kinds of enzymes released during fungal growth [82]. Apart from their effects on enzymes, D-glucosamine QA derivatives may also form complexes with vital metallic elements in the fungi to block or decrease fungal growth.

2.3. Antiviral activities

The virucidal capacity of Zephiran (alkyl-dimethylbenzylammonium chloride) against various types of viruses was summarized by Klein and Deforet as early as 1983 [27]. Zephiran effectively inactivates lipid-containing (enveloped) viruses such as vaccinia virus, and some non-lipid-containing (non-enveloped) viruses such as reovirus, and bacteriophages. However, it is ineffective against smaller non-lipid-containing viruses such as picornaviruses. In some studies, QACs were found to possess antiviral properties against enveloped *Herpes simplex* virus (HSV)-1 [83], [84]. The virucidal mechanism of QACs for lipophilic enveloped viruses appears to involve disruption or detachment of the viral envelope with subsequent release of the nucleocapsid. Disruption of the viral envelope may be attributed to the higher affinity of enveloped viruses for QAC through hydrophobic interactions. However, further disruption of the released nucleocapsids by QACs was not observed. The differential virucidal activities of QACs on enveloped or non-enveloped viruses were also investigated in another study [10]; the QACs tested were virucidal against enveloped *Influenza A* (H1N1) virus, but were ineffective against non-enveloped

Poliovirus Sabin1. Several QA camphor derivatives have been evaluated against a broad range of influenza viruses [85], [86], [87], [88]. These compounds interfered with the viral fusion process and effectively inhibited influenza virus replication in cell cultures. Shiraishi et al. screened the Takeda chemical library for novel anti-HIV-1 agents and identified an anilide derivative with a QA moiety as a potent and selective small-molecule (C—C chemokine receptor type 5) CCR5 antagonist for inhibition of macrophage-tropic HIV-1 replication [89]. Although the exact mechanism has not been fully elucidated, the QA moiety was found to enhance CCR5 antagonistic activity.

The antiviral activities of QACs against enveloped viruses have gained widespread recognition. However, whether QACs are effective against non-enveloped viruses remains controversial. QACs have repeatedly been reported as effective against some specific non-enveloped viruses. In studies evaluating the virucidal activity of disinfectants against various viruses, QACs are virucidal against feline viral rhinotracheitis virus and feline herpesvirus, but ineffective against feline calicivirus, feline panleukopenia virus (a parvovirus), feline parvovirus, canine parvovirus, and foot-and-mouth disease virus [90], [91], [92], [93], [94]. The lethal activity of QACs on enveloped viruses is linked to detachment of the viral envelope. In non-enveloped viruses, QACs induce formation of nonstructural substances such as micelles but are not lethal to the viruses [95]. A meta-analysis on virus inactivation by chemical disinfectants indicates that QACs are relatively ineffective against human Norovirus [96]. Recently, a topical QA silane prepared by sol-gel reaction of an antimicrobial trialkoxysilane with an anchoring tetra-alkoxysilane (codenamed “K-21”) has been reported to inhibit the replication of enveloped and non-enveloped DNA and RNA viruses at non-toxic concentrations, including *HSV-1*, *Human Herpesvirus-6* and *Human Herpesvirus-7*. These viruses have the capability to establish life-long latency in humans and can be reactivated later in life [97]. Because reactivation of the immunosuppressive and neurotrophic *Human Herpesvirus-6* in human brain tissues can cause cognitive dysfunction, permanent disability or death, and may play a role in a subset of patients with chronic neurological conditions such as multiple sclerosis, mesial temporal lobe epilepsy, status epilepticus and chronic fatigue syndrome, there is an urgent need for more studies on the capability for the K-21 agent to inhibit the replication of *Human Herpesvirus-6 in vivo*.

2.4. Anti-MMP activities

Host-derived MMPs and cysteine cathepsins play a major role, among other factors, in compromising the durability of resin-dentin bonds in tooth-colored filling materials via enzymatic hydrolysis of the collagen matrix [98], [99]. During dentin formation, MMPs, a group of zinc- and calcium-dependent host-derived proteases, are highly active in enzymatic hydrolysis of the collagen matrix [100]. Inactive MMPs can be activated by mild acids during the dentin caries process [101]. Single nucleotide polymorphisms of *MMP2* and *MMP3* gene are involved in dental caries susceptibility [102], [103]. Dental adhesives containing 5 wt% 12-methacryloyloxy dodecyl pyridinium bromide (MDPB) exhibited 89% inhibition of soluble recombinant human MMP-9 and 90% inhibition of matrix-bound endogenous MMPs [104]. The inhibitory effect of MDPB on MMPs may contribute to the improved durability achieved by MDPB-containing adhesives. Since MDPB can be copolymerized with methacrylate resin comonomers in the adhesive-tooth interface, such MMP-inhibitors may keep inhibiting MMPs over time. The inhibitory effect of QA methacrylates on endogenous dentin MMPs has been attributed to prevention of the release of collagen degradation products, including cross-linked carboxyterminal telopeptide of type I collagen (ICTP) by MMPs and C-terminal crosslinked telopeptide of type I collagen fragments (CTX) by cathepsin K [105]. Although the exact mechanisms of the anti-MMP effects of QACs have not been elucidated, it has been hypothesized that cationic QACs can electrostatically act on the catalytic site of MMPs, thereby inhibiting MMP-catalyzed hydrolysis of the collagen matrix [106], [107].

Matrix metalloproteinases also play important roles in pulp tissue and periapical inflammation [108]. Bacterial lipopolysaccharide (LPS)-induced release of pro-inflammatory mediators elevate levels of MMP-1, -2 and -3 in acute pulpitis [109], [110]. Release of MMP-3 can activate other MMP members, mediate collagen degradation in the extracellular matrix, induce inflammation and promote angiogenesis [111]. MMP-13 is the major collagenase in dental pulp tissues and is effective in cleaving type II collagen. In periodontitis, MMP family members have been confirmed to be associated with severe tissue disorganization [112], [113], [114]. A recent clinical investigation identified higher levels of MMP-1, -2 and -9 in the periapical interstitial fluid of patients with apical periodontitis [115]. Hence, in addition to their antimicrobial properties, incorporation of QACs into sealers for root canal treatment has the potential to inhibit MMPs directly for controlling the severity of periapical inflammation.

3. QA-based antimicrobial surfaces

3.1. “Grafting onto” vs “grafting from” approaches

Tethering of functional polymers with QA functionalities is an effective means to impart antimicrobial properties to biomedical materials. Such antimicrobial surfaces are generally prepared via two major immobilization methods, the “grafting onto” and “grafting from” approaches [116] (Fig. 3).

Fig. 3

Immobilization methods to produce antimicrobial surfaces by covalent attachment. [116], Copyright 2009.

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In the “grafting onto” approach, polymer molecules from solution are directly immobilized on suitable surfaces. Although experimentally simple, such an approach suffers from the drawbacks of relatively low grafting density as a result of steric hindrance from the already-adsorbed/attached polymer chains. These polymers form mushroom-like structures and render potential reactive sites inaccessible, further limiting the grafting density [117]. Thus, only a small amount of the polymers can be immobilized onto the surface using the “grafting onto” approach. Furthermore, the reaction between the polymer end-groups and reactive groups on the substrate surfaces becomes less efficient as the molecular weight of the polymer in solution increases [118].

The “grafting from” approach, also known as “surface-initiated polymerization”, has attracted increasing interest in recent years due to its ability to produce grafted polymers with better control of polymer features including functionality, density and thickness of the grafted polymers. The surface is first modified with suitable initiators. This surface-bound initiator monolayer allows surface-initiated polymerization of monomers to produce functional polymers with optimal thickness and higher density. Steric hindrance is greatly reduced because of the addition of monomers to the end of the growing chains or to primary radicals. Matyjaszewski’s group investigated the differences in bactericidal efficacy between grafted-onto and grafted-from surfaces. Compared with the “grafting onto” approach, the “grafting from” approach produced surfaces with a higher charge density (10^{16} vs 6×10^{14} charges/cm²)

and a higher biocidal efficacy ^[119] (Fig. 4). Based on the “grafting from” approach, many polymerization techniques including conventional free radical polymerization (FRP) and controlled radical polymerization (CRP) have found widespread use for the synthesis of antimicrobial surfaces.

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Fig. 4

Difference in biocidal activity of surfaces prepared by the “grafting onto” and the “grafting from” approaches. QA: quaternary amine group. ^[119], Copyright 2008.

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3.2. Controlled radical polymerization

Conventional FRP is a type of instantaneous chain growth polymerization involving chain initiation, propagation and termination ^[120], ^[121]. The biggest drawback to this technique is the large dispersity and poor control over molecular mass. Free radical polymerization techniques usually produce final products with a broader distribution of polymer chain length and/or equivalent degree of polymerization and functional group density due to unavoidable, fast radical–radical termination reactions ^[122].

Nevertheless, the compliant nature of FRP, including its versatility in monomer selection, relatively mild polymerization conditions and tolerance to many different solvents (such as water) and impurity, makes this polymerization technique one of the most widely used processes for preparing QA-based polymers from the perspective of industrial production and applications.

Over the last two decades, the advent of CRP techniques enables precise control over macromolecular structure, order and functionality, which are important considerations for emerging biomedical designs. In the following sections, the major surface-initiated controlled radical polymerization (SI-CRP) techniques will be discussed. [Table 3](#) summarizes the typical antimicrobial surfaces immobilized with QA moieties that have been prepared using SI-CRP.

Table 3

Typical antimicrobial surfaces immobilized with QA moieties prepared via surface-initiated controlled radical polymerization (SI-CRP).

Substrate	Grafted monomer	SI-CRP technique	Microorganisms tested	Refs.
Glass, filter paper	DMAEMA	SI-ATRP	<i>E. coli</i> , <i>B. subtilis</i>	[123]
Glass, silicon wafer	DMAEMA	SI-ATRP	<i>E. coli</i>	[124]
Glass	DMAEMA, TMSPMA	SI-ATRP	<i>E. coli</i>	[119]
Glass	DMAEMA	SI-ATRP	<i>A. niger</i>	[125]
Glass, PDMS, silicon wafer	DMAEMA, NIPAAm	SI-RAFT	<i>E. coli</i> , <i>S. aureus</i>	[126], [127]
Cellulose paper	DMAEMA	SI-RAFT	<i>E. coli</i>	[128]
Stainless steel	DMAEMA	SI-NMP	<i>E. coli</i> , <i>S. aureus</i>	[129]
Stainless steel	EA, PTEA, 8QA	SI-NMP	<i>E. coli</i> , <i>S. aureus</i>	[130]
Stainless steel	4VP	SI-ATRP	<i>D. desulfuricans</i>	[131]
Stainless steel	DMAEMA	SI-ATRP	<i>D. desulfuricans</i>	[132]
Stainless steel	BPEA, FAc	SI-ATRP	<i>S. aureus</i>	[133]
Stainless steel	DMAEMA	SI-ATRP	<i>D. desulfuricans</i>	[134]
Fe ₃ O ₄ magnetite nanoparticle	DMAEMA	SI-ATRP	<i>E. coli</i>	[135]
Fe(acac) ₃ magnetite nanoparticle	4VP	SI-ATRP	NM	[136]
Titanium	HEMA	SI-ATRP	<i>S. aureus</i>	[137]
Gold, Si/SiO ₂ surface	SPMA	SI-ATRP	<i>P. aeruginosa</i>	[138]
Silicon wafer	DMAEMA	SI-ATRP	<i>P. sp</i>	[139]
Silicon wafer, gold-coated silicon wafer, glass, cellulose acetate, silicon nanowire array, PU, PDMS, stainless steel	P(AA-co-Ada), PAH	SI-RAFT	<i>E. coli</i> , <i>S. aureus</i>	[140]
Silicon rubber	AAm	SI-ATRP	<i>S. aureus</i> , <i>E. coli</i>	[141]
Silicon rubber	AAm	SI-ATRP	<i>S. aureus</i> , <i>S. salivarius</i> , <i>C. albicans</i>	[142]
Silicon nanowire array	DMAEMA	SI-ATRP	<i>E. coli</i>	[143]
Silicon catheter	DMAEMA	SI-ATRP	<i>S. aureus</i>	[144]
PVDF membrane	DMAEMA	SI-RAFT	<i>E. coli</i>	[145]

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Among the different CRP techniques that are available, atom transfer radical polymerization (ATRP) has been the most extensively studied [124], [155], [156] and reviewed for the preparation of a wide variety of polymeric materials [157], [158], [159], [160]. The first antimicrobial surface with QA moieties prepared via surface initiated-ATRP (SI-ATRP) was reported by Matyjaszewski's group [123]. In that study, 2-(dimethylamino)ethyl methacrylate (DMAEMA) was polymerized directly onto filter paper via SI-ATRP.

Subsequent quaternization of the amino groups of p(DMAEMA) generated a high concentration of QA groups on the polymer-modified surface (Fig. 5). These modified surfaces exhibited substantial antimicrobial activities against *Escherichia coli* and *Bacillus subtilis*. The authors found that biocidal activity increased with the density of available QA groups on the modified surface. Different functional monomers have subsequently been polymerized via SI-ATRP followed by quaternization to immobilize QA groups onto the polymer surfaces. Techniques involving SI-ATRP are excellent for preparing antimicrobial surfaces with QA groups because of their: (1) chemical versatility and compatibility with functional groups and monomers, (2) tolerance against a relatively high degree of impurities, and (3) relatively easy synthesis of surface-immobilizable initiators. Nevertheless, it is challenging to achieve controlled polymerization of pyridine-containing monomers, since the latter can complex or react with the metal catalysts. In addition, residual traces of catalysts are difficult to remove, which may result in undesirable toxicity in biomedical materials.

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[Fig. 5](#)

Representative quaternary ammonium-based antimicrobial coatings prepared by controlled radical polymerization (CRP). ATRP: atom transfer radical polymerization; CTA: chain transfer agent; DMAEMA: 2-(dimethylamino)ethyl methacrylate; NMP: nitroxide-mediated polymerization; RAFT: reversible addition fragmentation chain transfer; SINMP: surface initiated nitroxide-mediated polymerization. [123], Copyright 2004.

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Reversible addition fragmentation chain transfer (RAFT) polymerization was introduced by Moad's group [161], [162]. This technique possesses several advantages, the most important being the ability to synthesize well-defined polymers with various polar and nonpolar monomers under mild polymerization conditions. In addition, implementation of RAFT polymerization is relative simple and versatile by adding an appropriate chain transfer agent (CTA) into a conventional FRP system. Usually, the same monomers, initiators, solution and temperatures may be used. Hence, RAFT polymerization has great potential for preparation of antimicrobial coatings [163]. The most critical issue for successful RAFT polymerization is the selection of a suitable CTA. Commonly-used CTAs include dithioesters, xanthates, dithiocarbamates and trithiocarbonates [164]. Antimicrobial surfaces with QA moieties have been successfully prepared via surface initiated-RAFT (SI-RAFT) polymerization. For example, cellulose filter paper was polymerized with DMAEMA via SI-RAFT and further quaternized [128] (Fig. 5). When the modified materials were exposed to *E. coli*, cellulose fibers with the highest degree of quaternization or quaternized with the shortest alkyl chains exhibited more potent antimicrobial activities. Recently, Wang et al. reported the synthesis of a temperature-triggered, recycable bactericidal and antifouling surface [126], [127]. At a temperature above the lower critical solution temperature (LCST), the biomaterial surface is able to capture and effectively kill bacteria due to the presence of quaternized p(DMAEMA). Remarkably, the surface is capable of releasing the adhered bacteria corpses when the temperature is reduced to below the LCST. Moreover, the surface maintains its self-cleaning and bactericidal properties

by simply washing with cold water and has good biocompatibility. This functionalized coating shows potential for multiple medical applications, including drug delivery, surface modification and tissue engineering. However, a major drawback of RAFT polymerization is that different monomers require specific CTAs, which are usually not commercially available. For example, methyl methacrylate (MMA) requires the use of a dithiobenzoate, whereas vinyl acetate can only be polymerized in the presence of a xanthate [165], [166].

Nitroxide-mediated polymerization (NMP), the simplest CRP technique, is accomplished by introducing a free nitroxide to the conventional FRP process. This type of polymerization is based on a reversible termination mechanism between the propagating radical and the nitroxide. The nitroxide acts as a control agent to yield alkoxyamine as the predominant species [167]. Owing to the living nature of NMP, this method can be applied to surface-initiated polymerization (SI-NMP) for preparing antimicrobial surfaces. In a typical SI-NMP process, binding of the initiator depends on the chemical nature of the surface, which may be achieved via covalent bonding, electrostatic interaction or hydrogen bonding. Ignatova et al. reported a two-step “grafting from” method for preparing antimicrobial stainless steel surfaces by sequential cathodic electrografting of an alkoxyamine-containing acrylate with SI-NMP of styrene and DMAEMA, followed by quaternization of the grafted polymers [129] (Fig. 5). The modified surface possesses significant antibacterial activities against both *Staphylococcus aureus* and *E. coli*. The thickness and hydrophilicity of the immobilized polymers may also be tailored by SI-NMP. Because no additional catalysts are required, this avoids the need for additional purification and reduces the chance to introduce impurities. Konn et al. used a similar alkoxyamine anchoring strategy with synthetic laponite clay platelets [154]. In this process, a quaternary ammonium alkoxyamine initiator is first intercalated into the clay galleries by cation exchange. Then, NMP of styrene was initiated from the surface of the functionalized clay platelets to obtain well-defined, ionically-bonded polystyrene chains with narrowly-distributed molecular weight. However, relatively high polymerization temperatures may cause problems when thermally-sensitive monomers are employed. In addition, the selection and synthesis of suitable nitroxides increase the workload in preparing antimicrobial coatings. Nitroxide-mediated polymerization may also be accomplished using the “grafting onto” approach. However, this synthesis pathway is not as popular due to steric hindrance of the grafted polymers.

3.3. Ring-opening polymerization

The history of ring-opening polymerization (ROP) dates back to the 1900s and presently, many polymers of industrial importance are produced using ROP [168]. In the ROP process, the terminal end of a polymer chain acts as a reactive center, which may be radical, anionic or cationic. Cyclic monomers can polymerize by opening the ring system using metal catalysts [169]. Depending on the nature of the initiator and catalyst, ROP may proceed via radical, cationic or anionic reaction mechanisms [170]. Examples of antimicrobial surfaces with QA moieties prepared by ROP have also been reported in the literature (Table 4). Wynne’s group prepared a series of quaternary/polyethylene glycol (PEG) copolyoxetanes via cationic ROP; these copolyoxetanes possessed biocidal activities against *E. coli*, *S. aureus* and *Pseudomonas aeruginosa* [171], [172]. The authors found that both linear charge density and quaternary alkyl chain length affected antimicrobial efficacy, hemolytic activity and cytotoxicity. Other workers concentrated on the synthesis of QA-functionalized surfaces/materials with dual antimicrobial actions [173], [174]. Nitric oxide (NO)-releasing, QA-functionalized silica nanoparticles are synthesized by first tethering glycidyltrialkylammoniumchloride (GTAC) via ROP. This is followed by functionalization of the secondary amine with N-diazeniumdiolate, the NO donor (Fig. 6).

Table 4

Typical biomaterials grafted with QA-based polymers via ring-opening polymerization (ROP).

Products	Microorganisms tested	Refs.
PEG/quaternary copolyoxetanes	<i>S. aureus</i> , <i>E. coli</i> , <i>P. aeruginosa</i>	[171], [172]
Polycarbonate hydrogels	<i>S. aureus</i> , <i>E. coli</i> , <i>P. aeruginosa</i> , <i>C. albicans</i>	[176]
QPGMA polymers	<i>E. coli</i> , <i>S. aureus</i> .	[177]
PAMAM dendrimers	<i>S. aureus</i> , <i>P. aeruginosa</i>	[173]
Silica nanoparticles	<i>S. aureus</i> , <i>P. aeruginosa</i> .	[174]

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[Fig. 6](#)

Representative biomaterials grafted with QA-based polymers via ring-opening polymerization (ROP). A. Synthesis of nitric oxide (NO)-releasing QA-functionalized silica nanoparticles. B. Schematic illustration of nitric oxide (NO)-releasing QA-functionalized silica nanoparticles. [174], Copyright 2012.

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Compared with nanoparticles with NO release or QA functionalities only, combining NO release with QA functionalities on the same nanoparticle resulted in augmented bactericidal efficacy against *S. aureus*. Nitric oxide is an endogenously synthesized small molecule involved in vital cellular functions and exhibits antimicrobial effects against a wide range of organisms. Hence, exogenous NO donors are promising bactericidal agents [19]. The development of novel monomers and catalysts enables more precise control over the molecular weight, structure and configuration of the polymers. In some cases, ROP and CRP techniques may be implemented together. For example, Leng et al. reported the synthesis of copolymer micelles of poly(caprolactone)-poly(quaternary ammonium salt) by a combination of ROP and ATRP. These copolymer micelles do not only possess antibacterial ability but may also be employed as carriers for antibacterial drugs [175].

3.4. Click chemistry

Click chemistry has recently emerged to become one of the most powerful tools in syntheses involving biomedical and chemical applications, including organic synthesis, medicinal chemistry, surface and polymer chemistry, drug discovery and chemical biology. The term “click chemistry” was coined by Sharpless and coworkers in 2001, and refers to a method for attaching a substrate of choice to specific biomolecules (site-specific bioconjugation) [178]. Click reactions are more broadly defined contemporarily as those that meet the criteria of ready availability of starting materials and reagents, high

efficiency under mild conditions, regio- and stereo-selectivity, high yield, minimal by-products and limited side reactions. The aforementioned characteristics may be achieved by reactions possessing a high thermodynamic driving force (usually greater than 20 kcal mol⁻¹). The most common methods for incorporating clickable groups into polymer chains include: (1) the use of functional initiators or transfer agents, (2) direct polymerization of functional monomers yielding pendant functionality and (3) post-polymerization transformation of end groups, each of which yielding terminal functionality [179].

Recently, the application of click techniques has found widespread use in the synthesis chemistry and there are a number of comprehensive reviews highlighting these exciting approaches [180], [181], [182], [183], [184]. Some of the most commonly used click reactions for creating antimicrobial surfaces with QA moieties, including Cu-catalyzed azide-alkyne cycloaddition (CuAAC), Cu-free click cycloaddition and thiol-ene reaction are illustrated in Fig. 7.

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Fig. 7

Schematic illustration of reactions that best meet the criteria for a click reaction. A. Cu(I)-catalyzed azide-alkyne cycloaddition (CuAAC); B. Cu-free click cycloaddition; C. thiol-ene reaction. [183], Copyright 2011.

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The quintessential example of click chemistry, CuAAC, has generated the highest interest for producing QA antimicrobial surfaces [185], [186]. In a CuAAC reaction, [3 + 2] cycloaddition occurs between an organic azide and a terminal alkyne to produce Cu(I)-catalyzed 1,4-disubstituted 1,2,3-triazole [187]. In general, three key processes are involved in a CuAAC reaction: (1) initial formation of a 5-triazolyl copper intermediate, (2) coordination of the intermediate to organic azide and reaction between the nucleophilic carbon on the Cu(I) acetylide and the electrophilic terminal nitrogen on the azide, (3) ring contraction of the metallocycle and subsequent dissociation of the product to regenerate the catalyst. The reactions can accommodate a wide variety of functional groups and can proceed in many solvents (e.g. water), a wide range of pH values and over a broad temperature range. The reaction rate of CuAAC is more than 10⁷ times faster than conventional reactions, which means that CuAAC may proceed efficiently at ambient temperature. It does not take long for chemists to realize the practicality and reliability of CuAAC in creating QA-based antimicrobial surfaces. For example, Ganewatta et al. recently produced QA-modified antimicrobial and biofilm-disrupting surfaces via CuAAC between surface-immobilized azide groups and the alkyne moiety on QACs [188] (Fig. 8). Other antimicrobial surfaces are produced by post-polymerization modification, via quaternization of tertiary amines to QA groups [189], [190], [191]. These reactions often involve production of cations of p(DMAEMA), p(2-vinylpyridine) (2-VP) and p(4-VP).

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[Fig. 8](#)

Representative quaternary ammonium-based antimicrobial surfaces prepared by Cu-catalyzed azide–alkyne cycloaddition (CuAAC). A. Synthesis of antimicrobial surfaces. B. Stained (live/dead stain) surfaces after 24 h of incubation with *Staphylococcus aureus* and *Escherichia coli*. S1, pristine surface; S3, quaternary ammonium-based surfaces. Cells with green fluorescence indicate live bacteria colonizing the surface, while dead cells exhibit red fluorescence. C. Schematic representation of the antimicrobial activity of the QA surfaces. [188], Copyright 2015. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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An inherent limitation of CuAAC is the potential toxicity of catalyst Cu(I). To address this issue, Cu-free click chemistry has been developed [192]. Bertozzi et al. used strained alkynes (cyclooctynes) to facilitate strain-promoted cycloaddition of alkynes and azide (SPAAC) without a toxic catalyst [193], the reaction rate of which may be tuned by substituents on the cyclooctyne group. Copper-free click chemistry has attracted increasing interest in the fast-growing field of polymer and materials science because of its non-toxicity and biocompatibility. Applications of this strategy to QA surface modification have also been reported [194]. In that study, quantum dots were first modified with dibenzocyclooctynes (DBCO) to obtain DBCO-modified quantum dots. This was followed by SPAAC of the DBCO-modified quantum dots to azide-modified quaternized p(DMAEMA). Quaternary ammonium functionalized quantum dots possessed good antibacterial activity against *E. coli* and *S. aureus*, and exhibited no cytotoxicity on mammalian cells (Fig. 9).

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[Fig. 9](#)

Representative quaternary ammonium-based antimicrobial surfaces prepared by Cu-free click cycloaddition. A. Schematic of the synthesis of quaternary ammonium-functionalized quantum dots (QA-QDs). B. Representative scanning electron microscopy images of *Escherichia coli* incubated without (A–C) and with QPA-QDs for 20 min (D–F). C. Optical phase contrast images of cells double-stained with acridine orange and propidium iodide (AO/PI), after culturing with HepG2, A549, and HUVEC–C cells for 20 min with QPA-QDs (400× magnification). Cells with green fluorescence indicate live cells, while dead cells displayed red fluorescence. [194], Copyright 2016. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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Thiol-ene reaction has also been used for surface functionalization due to its efficiency, simplicity and high degree of conversion under mild conditions. Attachment of a molecule with a sterically accessible alkene or thiol group to a surface of interest enables surface functionalization via thiol-ene reaction. Tian et al. synthesized QA-functionalized antimicrobial wool fabric through thiol-ene reaction. The wool fabric was first treated with tris(2-carboxyethyl)phosphine to produce thiol groups for subsequent reaction with the C=C groups of a QA salt [195]. The antimicrobial efficiency of the modified fabric against *E. coli* and *S. aureus* was 86.3% and 90.1%, respectively. In radical initiated polymerization, three distinct processes including initiation, polymerization and termination are involved in thiol-ene reaction [196]. In an ideal thiol-ene reaction, the combined propagation and chain transfer reaction proceed at equivalent rates and one thiol reacts with one-ene to produce an addition product. This inherent nature of the reaction results in homogenous and uniform end products. Photochemistry may also be used to initiate the thiol-ene reaction. For example, Wen et al. synthesized a series of QA-functionalized hyperbranched polyglycerols via ultraviolet light-initiated thiol-ene click chemistry [197] (Fig. 10). Compared with other methodologies such as thermal and redox initiation, the use of photoinitiation is particularly attractive because it allows both spatial and temporal control over the progress of the reaction.

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Fig. 10

Synthesis of sulfobetaine/quaternary ammonium modified, hyperbranched polyglycerols (HPGs) via ultraviolet light-initiated thiol-ene “click” chemistry. [197], Copyright 2016.

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4. Orthopedics-related materials

Despite the adoption of stringent sterilization and aseptic procedures, biomedical material-associated infections remain a critical challenge in orthopedics. The incidence of peri-prosthetic infection is 0.5–5%, depending on the surgical site and the procedure undertaken [198]. Among the diverse microorganisms present, gram-positive pathogens are predominant in deep infections, predisposing individuals to pain, high treatment cost and reduced treatment success. Hence, biomedical materials with anti-infective properties are highly desirable in orthopedics to prevent potential infections, and to serve as local delivery systems for antimicrobial agents to treat deep bone infections (osteomyelitis). Controlled release of antibiotics such as gentamicin from bone cements and beads represent the current gold standard for local antibiotic delivery, for preventing infections after total joint replacement and for treating osteomyelitis [199], [200], [201]. Non-degradable poly(methyl methacrylate) (PMMA) bone cements and spacer beads loaded with antibiotics can release drugs from the cement over time with a high initial release rate followed by sustained release. Nevertheless, their use is also associated with drawbacks such as unpredictable long-term efficacy, potential antibiotic resistance and local tissue toxicity. These issues may compromise cell and bone regeneration and implant osseointegration [202], [203]. The effectiveness of such devices is strongly dependent on the release rate of the loaded drugs [204]. In addition, long-term low-level release below the minimum inhibitory concentration of antibiotics following the “initial burst release” may contribute to the development of antibiotic-resistant bacteria strains.

Several publications support that the use of QACs in providing stable and permanent antimicrobial properties to bone cements ([Table 5](#)). Punyani et al. developed QA-based PMMA bone cements using a free radical bulk polymerization technique [\[205\]](#). The bone cements exhibit a broad spectrum of contact-killing antimicrobial properties against *E. coli* and *S. aureus*. The modified cements and their eluants elicited negligible immune response and chronic inflammation in the host tissues [\[206\]](#). These materials effectively kill bacterial cells attached to the cement surface. Incorporation of less than 15 wt% of these QACs to the bone cements results in no alteration of mechanical properties; the methacryloxy functionality renders these QACs polymerizable with methacrylate bone cements. Compared with linear polymers, dendrimers have many advantages as antimicrobial agents. The controlled synthetic process produces final molecules with well-defined architectures. The specific spherical-shaped structure provides high surface functional group concentration [\[295\]](#). Hyperbranched QAC-functionalized tripropylene glycol diacrylate (TPGDA) dendrimers have been incorporated into bone cements due to their large number of QA functionalities [\[208\]](#). The modified cement can kill up to 10^8 CFU/mL of common hospital-acquired bacteria such as *E. coli*, *S. aureus* and *P. aeruginosa* by contact-killing, with sustained bactericidal activity over a 30-day period. These antimicrobial properties render the modified bone cement promising for combating infections related with prostheses and surgeries.

Table 5

Quaternary ammonium-based biomedical materials, QA-based nanoparticles, and the combination of QACs and nanomaterials.

Applications		QACs	Microorganisms tested	Antibacterial methodologies	Detailed
Orthopedics-related materials	Bone cements	QADMA	<i>E. coli</i>	Agar diffusion test, bacteria viability (colony counts), SEM	QA cen killi proj rele age
		QAMA	<i>E. coli, S. aureus</i>	Agar diffusion test, bacteria viability (colony counts), SEM	Incl pro with proj poly and mec with cyto
		TPGDA	<i>E. coli, S. aureus, P. aeruginosa</i>	Bacteria viability (colony counts), agar diffusion test	The stru for acti the of b

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Quaternary ammonium polyethylenimine (QPEI) nanoparticles and quaternized chitosan derivatives are two examples of QA-functionalized fillers incorporated into PMMA bone cements. The QPEI nanoparticles have been used in various dental materials (see Section 6) because of their excellent antibacterial activities, mechanical properties and biosafety. Bone cements loaded with the nanoparticles retain effective antibacterial activity after a 4-week aging period, without altering the biocompatibility or

mechanical properties of PMMA [209]. From a clinical perspective, PMMA bone cements loaded with 26 wt% hydroxypropyltrimethyl ammonium chloride chitosan (HACC), a quaternized chitosan derivative, prevent bacterial biofilm formation of antibiotic-resistant bacteria strains (methicillin-resistant *S. aureus* standard strains and clinically-isolated methicillin-resistant *Staphylococcus epidermidis*) and down-regulate virulence-associated gene expressions on the cement surface [209]. The HACC-loaded PMMA was found to induce better stem cell proliferation, osteogenic differentiation and osteogenesis-associated genes expression when compared with gentamicin-loaded PMMA because local release of antibiotics in bone cements suppresses osseointegration [210] (Fig. 11).

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Fig. 11

A. Cytoskeletal morphology of human mesenchymal stem cells (hMSCs) exposed to different polymethyl methacrylate (PMMA)-based bone cements. B. Relative alkaline phosphatase activity (indicative of bone forming potential) of hMSCs after 6, 10, and 14 days of culture. For each time period, columns connected with a horizontal bar are not significantly different ($P > 0.05$). C. Representative images showing hMSCs with positive alkaline phosphatase staining on the four PMMA-based bone cements at day 14. PMMA-C: chitosan-loaded PMMA; PMMA-G: gentamicin-loaded PMMA; PMMA-H: hydroxypropyltrimethyl ammonium chloride chitosan (HACC)-loaded PMMA. [210], Copyright 2012.

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The favorable results achieved by HACC-loaded PMMA may be attributed to improved surface roughness and wettability, increased porosity and better attachment and spreading of human-marrow-derived mesenchymal stem cells on the cement surface. In a rabbit model, HACC-loaded PMMA inhibits *in vivo* bone infections induced by methicillin-resistant *S. epidermidis*, with no bone lysis, periosteal reactions, cyst formation and sequestration of non-vital bone [211], [212]. The quaternized chitosan derivative may also be immobilized on titanium implant surfaces via silanization with aminopropyltriethoxysilane [215]. Incorporation of silyl groups enables covalent attachment of the quaternized chitosan derivative on the metal or hydroxylated surfaces, while the QA group exerts long-term antibacterial activity. Titanium surfaces modified with HACC prevent bacteria attachment *in vitro* and reduce infection *in vivo*. The coated titanium implants are well-integrated with the surrounding bone without bacterial growth. Nevertheless, clinical trials and FDA approval are required before clinical translation and commercialization of this technology can be successfully achieved.

Incorporation of both contact-based biocides such as QACs, and release-based biocides such as AgNPs [296], [297] or silver bromide (AgBr) NPs [298] produces surfaces with dual-functional antibacterial activities. This strategy is of particular interest in orthopedic operations because of its unique antibacterial characteristics. Dual-functional coatings exhibit potent initial antibacterial properties due to the release of bioactive biocides and maintain long-term antibacterial activities via the immobilized QA groups. The first 6 h after surgical implantation is highly susceptible to bacterial invasion and surface colonization, and has been coined the “decisive period” [299]. A high initial burst release offers protection against the initial elevated risk of infection, while sustained contact-killing provides surveillance against subsequent latent infections.

5. Sutures and wound dressings

Wound management is a critical healthcare component that has attracted much global concerns. Sutures are used to approximate tissues to facilitate closure and healing of wounds [300]. Bacteria attachment and proliferation on suture materials are significant risk factors for wound infections [301]. Wound dressings generally consist of bilayer structures, including an upper dense “skin” layer and a lower spongy layer. Although dressings are designed to protect the wound against microorganisms [302], the wounded site in the vicinity of the dressing provides a favorable environment for bacterial growth. Intensive research has been conducted on antibacterial modification of wound dressings to prevent infections and provide an optimal milieu for tissue regeneration [303], [304]. These antibacterial materials are produced predominantly by electrospinning [305]. Sustained broad-spectrum antimicrobial activity is desirable for optimal wound healing because complex tissue interactions between cells, extracellular matrix molecules and soluble mediators are involved in the regeneration of damaged skin or other tissues. Previous studies reported addition of small molecule QACs such as benzalkonium chloride, *N,N,n,n*-didecyl-*N,N*-dimethylammonium chloride to polymer solutions for electrospinning of antibacterial nanofibers [306], [307]. Such methods suffer from drawbacks such as reduction in fiber diameter and a burst release of QACs. Compared with the attachment of QACs to a wound dressing through ionic absorption, immobilization of QA moieties on the dressing surface via covalent bonding provides long-term antibacterial protection and eradication of bacteria by contact-killing (Table 5). Various polymerization techniques including layer-by-layer assembly, click chemistry, FRP and CRP have been employed [308], [309], [310]. For example, Dan et al. reported the preparation of antimicrobial keratin fibers for medical applications through thiol-ene click chemistry [216]. The presence of QA moieties on keratin structures was confirmed with Raman and infrared spectroscopy. The modified sutures exhibit excellent antimicrobial activity, antistatic property and significantly improved moisture management capability. Sutures and dental floss that were coated with the QA compound “K21” demonstrated dose-dependent antibacterial activities against the periodontal pathogen *P. gingivalis* and the endodontic pathogen *Enterococcus faecalis* [217]. Liu et al. found that compared with traditional pad-dry-cure process, QA-functionalized cotton fabric prepared using a pad-dry process showed a relatively higher antibacterial efficacy [218]. The phenomenon may be attributed to increased hydrophobicity of the coated samples under high coating temperature in the pad-dry-cure process. Recently, Kang et al. reported improved antibacterial efficacy in antimicrobial cotton fibers that are chemically treated with glycidyltrimethylammonium chloride and AgNPs [219] (Fig. 12). In addition, the coated fibers exhibit increased moisture regain as a result of the enhanced hydrophilicity.

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Fig. 12

Representative scanning electron microscopy images of A. Untreated cotton; B. Cotton treated with silver nanoparticles (AgNPs). C. Cotton treated cotton with quaternary ammonium compound. D. Cotton treated with quaternary ammonium compound and AgNPs. [219], Copyright 2016.

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Attempts have also been made to incorporate QA groups into wound dressing materials. Attachment of QA groups to a polyurethane foam wound dressing [220] resulted in inhibition of biofilm development of three major wound pathogens (*S. aureus*, *P. aeruginosa* and *Acinetobacter baumannii*) both within the wound and in the wound dressing. Wound healing requires a moist and warm environment, and dressing materials with good air permeability and moisture absorption are desirable. Novel QA-containing polyurethane membranes used as wound dressings exhibit antibacterial activity against *S. aureus* and *E. coli* [221]. The modified antibacterial dressings exhibit good biocompatibility for fibroblasts and epidermal keratinocytes. More importantly, the modified dressings possess excellent water vapor transmission to prevent accumulation of exudates and decrease surface inflammation within the wounded area. Hydrogels based on synthetic or natural polymers may be used as wound dressings to locally deliver a wide range of bioactive agents in a controlled and sustained manner to regulate stem cells that are encapsulated within the three-dimensional polymer network [311]. Because of their biocompatibility, low immunogenicity and rapid hydrolysis under physiologic conditions, some hydrogels have been commercialized for clinical use [312]. Recently, a gelatin hydrogel grafted with an epoxy silicone QA salt exhibits excellent antibacterial property against Gram-positive and Gram-negative bacteria, including *S. aureus*, *E. coli* and *Bacillus subtilis* [63]. The quaternized polycarbonate hydrogels have been successfully synthesized via ROP [176]. In addition to their broad-spectrum antimicrobial activity, the gels showed fast degradation at room temperature (4–6 days). This makes them promising for incorporation into wound healing and implantable biomaterials. Pilakasiri et al. found that QA-modified chitin gels are promising candidates for treatment of full-thickness open wounds, with better wound healing than a clinically available dressing [223].

6. Dental materials

Dental caries is one of the most prevalent bacteria-related human infectious diseases [313]. During the development of dental caries, acidogenic and aciduric bacteria such as *Streptococcus mutans* accumulate on teeth and/or dental fillings and cause demineralization of enamel and dentin via acid generation [314]. Following demineralization, salivary proteases or endogenous peptidases such as MMPs and cysteine cathepsins induce degradation of the demineralized collagen matrix [315]. To address the growing need for antibacterial dental materials, a useful approach is to permanently copolymerize QACs with resin materials by forming covalent bonds with the polymer network. Such stable non-leaching antibacterial materials can provide long-term protection via contact of the microorganisms with the biocidal surface, without sacrificing the mechanical properties and polymerization behavior of the original non-antibacterial resin formulations [70], [226]. In particular, QA-based resin materials exhibit good biocompatibility that is evidenced by minimal toxicity, allergenicity and tissue irritability [51]. Based on the aforementioned advantages, QACs are considered highly desirable for managing and combating dental caries.

The first synthetic QAC to be incorporated in antibacterial dental materials is 12-methacryloyloxy dodecyl pyridinium bromide (MDPB) [66]. Polymerized dental adhesives containing MDPB significantly exhibit contact-killing effects on *S. mutans* when these bacteria come into contact with the specimens' surface [253]. Composites incorporated with MDPB exhibit long-term antibacterial activity without eluting antibacterial components. Dental materials containing methacryloxylethyl cetyl ammonium chloride (DMAE-CB) exhibit strong, fast and long-term killing effects [58], [261] (Fig. 13). Adhesives incorporating DMAE-CB suppress the expression of glucosyltransferase genes which contribute to plaque biofilm accumulation [259], [260]. The application of QACs in dental materials has been published in several reviews [316], [317]. These discoveries led to the development of various kinds of non-releasing

antibacterial dental materials including resin composites, adhesives, glass ionomer cements and resin-modified glass ionomer cements, root canal sealers, pit-and-fissure sealants, pulp capping materials, acrylic resin dentures, orthodontic retainers, denture lining materials and dental implants ([Table 5](#)).

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[Fig. 13](#)

Antibacterial activity of polymerized dental adhesives containing MDPB or DMAE-CB. A-C. Representative confocal laser scanning microscopy images of *Streptococcus mutans* adhered on the control adhesive (A), adhesive containing MDPB (B) and adhesive containing DMAE-CB (C) after 24-h incubation. Bacteria with integral membranes were stained with green fluorescence and those with compromised membranes were stained with red fluorescence. D. Fluorescence intensity values of the two channels for adhesives derived from the three groups. E. Schematic representation of polymerized adhesive containing DMAE-CB. F-H. Representative scanning electron microscopy images of *Streptococcus mutans* biofilms on the control adhesive (F), adhesive containing MDPB (G) and adhesive containing DMAE-CB (H) after 4-h incubation. I-K. Representative scanning electron microscopy images of *Streptococcus mutans* biofilms on the control adhesive (I), adhesive containing MDPB (J) and adhesive containing DMAE-CB (K) after 24-h incubation. [\[260\]](#), Copyright 2009. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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Over the last decade, researches on QACs have benefited from ground breaking advancements in organic and inorganic chemistry as well as materials science. These improvements have contributed to the introduction of strategies to develop innovative QACs with improved properties. Theoretically, antibacterial materials incorporated with more QACs provide better antimicrobial activities. However, the capability of a resin polymer network to copolymerize with QACs is limited (e.g. only 5 wt% MDPB and 3 wt% DMAE-CB are employed in adhesives). Incorporating too high concentrations of these mono-methacrylates (i.e. QACs with one methacrylate group) beyond the polymerizable capability of the resin polymer network may adversely affect its structural and mechanical properties. To address these problems, QACs with two methacrylate groups have been developed for incorporation in antibacterial dental materials [\[57\]](#), [\[240\]](#), [\[244\]](#), [\[287\]](#), [\[318\]](#).

In the investigation of the structure-property relationship of QACs [\[67\]](#), [\[68\]](#), those with a chain length of 12 or 16 were found to possess higher antibacterial potency. Due to the high electron density of iodine, substitution of the halogen anions in QACs with iodine as the counter ion results in radiopaque antibacterial monomers [\[69\]](#), [\[241\]](#), [\[242\]](#), [\[243\]](#), [\[245\]](#).

Most of the reported QACs have only antibacterial effects. Apart from bacteria, fungi play an important role in oral cavity infections such as denture-related stomatitis. Co-colonization of *S. mutans* and *Candida albicans* in the form of mixed dental plaque biofilms can occur on the surface of removable orthodontic appliances or removable dentures [\[319\]](#), [\[320\]](#). Recently, quaternary ammonium methacryloxy silicate (QAMS) has been developed with both antibacterial and anti-fungal properties for incorporation into dental removable appliances fabricated from acrylic resin (i.e. PMMA) [\[288\]](#), [\[289\]](#), [\[290\]](#). In those *in vitro*

studies, acrylic resin containing 5 wt% QAMS exhibits contact-killing microbiocidal activities on *S. mutans*, *Actinomyces naeshlundii* and *C. albicans* single species biofilms. Thus, QAMS has the potential for fabrication of antibacterial/anti-fungal partial/full denture bases, as well as for preparation of removable orthodontic appliances and retainers.

Polymerization shrinkage stresses and strains are generally considered the most pertinent issues associated with the clinical use of resin-based dental filling materials. These stresses and resulting strains may contribute to crack propagation in the filling material, leakage along the tooth-filling interface, bacterial invasion and secondary caries [321], [322]. Burujeny and colleagues fabricated thiol-ene-methacrylate containing quaternary amine moieties with reduced polymerization shrinkage stress and strain and improved antibacterial effectiveness [247]. Reduction in polymerization shrinkage has been attributed to the nature of thiol-ene polymerization; delayed gelation produces more homogeneous structure with narrow glass transition temperature ranges and reduced brittleness in comparison with classic methacrylate resin systems [323], [324]. In addition, the presence of flexible thiol-ether linkages provide a better opportunity for exposure of reactive quaternary amine groups on the surface of the polymerized resin matrix, which, in turn, provides better bactericidal function.

Biomedical materials have evolved with the advent of nanotechnology, making it possible to develop antibacterial dental materials with QAC-functionalized nano-fillers. Quaternary ammonium polyethylenimine (QPEI) nanoparticles are effective against a variety of Gram-positive and Gram-negative bacteria, including clinical isolates of pathogenic bacteria and bacteria in contaminated water [325], [326]. The antimicrobial potency of these nanoparticles is attributed to the abundance of QA groups along its backbone. The QPEI is a synthetic, non-biodegradable, cationic polymer with primary, secondary and tertiary amine functionalities, which may be subjected to a wide range of chemical modifications to produce desirable physicochemical properties [16]. Because of the small size and large surface area of the nanoparticles, as well as their capability to release high concentrations of ions with low filler content, only a small amount is required for incorporation into dental materials. Incorporation of low concentration of these nanoparticles (1–2 wt%) into dental materials [229], [230], [282], [284], [327] provides potent and broad-spectrum antimicrobial effects. Silane-quaternary amine-based antimicrobials, generally consisting of an organic shell with antimicrobial functionality and a silica core with high colloidal stability, have been shown to be effective antimicrobial fillers for more than 40 years [328]. Reactive silane functionalized antimicrobial agents are resistant to microbial growth and biofilm formation. These antimicrobial agents also possess other benefits, including tunable surface functionality, low cytotoxicity and easily controllable size. Hence, they are widely used in the field of antimicrobials [329], [330], [331]. Silica-quaternary ammonium QPEI nanoparticles, prepared by surface modification of silica particles with QA groups, distribute more homogeneously in the polymeric coatings, with less nanoparticle aggregation when compared with QPEI nanoparticles [332]. For example, quaternary ammonium epoxy silicate nanoparticles applied as QA compounds conjugated to organosilanes and silica nanoparticles, and also to epoxy silicates, were synthesized by a silane-based sol-gel reaction and incorporated into an epoxy resin-based root canal sealer. The sealer exhibited antimicrobial activities against *E. faecalis* biofilms [281]. Resins incorporating functionalized silica nanoparticles possess sufficient antimicrobial activity as well as high strength and modulus of elasticity values even at low filler concentrations [333].

Dental materials with both QACs and nano-sized antibacterial agents incorporated into one system operate with dual functions [334]. For example, incorporation of both QACs and AgNPs can effectively kill bacterial both on (contact-based killing) and away from the surface of the materials (release-based killing) [268], [269]. The AgNPs are small enough (mean diameter 40 nm) to infiltrate dentinal tubules to

kill residual intratubular bacteria [270]. Incorporation of nanoparticles of amorphous calcium phosphate (NACPs) into QAC-based dental materials bestows those materials with remineralization capabilities for reversing the tooth decay process [335]. The QAC-based nanocomposites and adhesives containing NACPs possess “smart” properties by increasing Ca and P ion release at low cariogenic pH values, when these ions are most needed for inhibiting caries, neutralizing the demineralization of enamel and dentin by lactic acid, and preventing caries formation [232]. However, one should be careful in balancing the antibacterial effects of those nano-fillers with their cytotoxicity due to latent nanoparticles-related toxicity.

Although *in vitro* models have been adopted by researchers for initial screening of new materials and techniques for developing QACs and to justify their potential for clinical applications, there are other *in vivo* factors that cannot be duplicated in *in vitro* studies. To date, the majority of the work performed on the antimicrobial properties of QACs is *in vitro* nature. Although the recent use of multi-species oral biofilm models has contributed to the understanding of intraoral microbial adhesion and biofilm formation, these models are unlikely to simulate the variability and *in vivo* dynamics of dental plaque biofilms. For example, the antibacterial and immunoregulatory effects of antimicrobial peptides such as α - and β -defensins, and the cathelicidin LL-37 in the oral cavity, as well as histatins derived from the saliva cannot be neglected [336], [337]. Other factors include the role of saliva in slowing down tooth decay by neutralizing plaque acids [338]. In addition, saliva-derived protein films can be adsorbed on dental restoration surfaces and provide anchoring sites for bacteria [339], block functional antibacterial groups on the material surfaces, thereby reducing the antibacterial activity of QA-based materials. Indeed, several publications show that the presence of a saliva-derived protein film (i.e. pellicle) on the surface of QA-based materials reduce their bactericidal effects [224], [340]. Because pre-coating of salivary pellicles on resin surfaces significantly decreases the antibacterial effects of polymerized antibacterial resins, *in vivo* and *in-situ* models are highly desirable for evaluating treatment outcomes, which, unfortunately, are desperately lacking. Animal models have been developed for comparing the functional efficacy of QA-based dental materials. Generally, those animal models were designed to examine: a) the biocompatibility of the materials (pulp response and reparative dentin formation) [341]; b) the presence of bacteria within the intraradicular dentin [254]; c) bonding ability using QA-based dental adhesives [256] and restorative materials. Using a rat tooth cavity model, QA-based NACP-containing nanocomposites and adhesives were found to combat oral pathogens and biofilms effectively and facilitate healing of the dentin-pulp complex [234] (Fig. 14).

[Fig. 14](#)

Evaluation of antibacterial and remineralizing nanocomposite and adhesive containing nanoparticles of amorphous calcium phosphate (NACP) and dimethylaminododecyl methacrylate (DMADDM) in a rat tooth cavity model. A. Schematic representation of rat tooth cavity model. B. Rat right and left molar teeth were used. C. Occlusal cavity was prepared. D. Cavity was restored with adhesive and nanocomposite and light-cured. E-H. Histological analyses of rat tooth cavities at 8 days in the control group (E and F) and the DMADDM + NACP group (G and H). The same four groups at a higher magnification. The control group exhibited disruption of the odontoblast layer associated with a medium inflammatory response in the pulp. Blood vessels are observed and a thin layer of tertiary dentin can be identified. The DMADDM + NACP group has normal pulp tissues, with much thicker tertiary dentin (a type of reparative dentin) than the control. Stars indicate areas with inflammatory cell infiltration. I and J. Representative hematoxylin and eosin-stained images at 30 days in control group (I) and the DMADDM + NACP group (J). Stars indicate areas with inflammatory cells. Blood vessels are indicated by arrows. The control group exhibits slight inflammatory responses. The DMADDM + NACP group shows normal pulp tissue without inflammatory response, and greater tertiary dentin thickness. [234], Copyright 2014.

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It must be pointed out that data derived from animal studies are somewhat different from the clinical effects experienced by humans. Apart from differences in structural characteristics between *in vitro* and *in vivo* biofilms, the presence of host defenses such as antimicrobial peptides derived from saliva is seldom taken into account in *in vitro* multi-species biofilm models [342]. Plaque biofilm profiles vary among individuals because they are modulated by different environmental factors as well as quorum sensing signals derived from adjacent microorganisms [343], [344], [345]. These confounding factors may temper the efficacy of antimicrobial polymers *in vivo*. In the contemporary world of evidence-based medicine and dentistry which demands the conscientious, explicit and judicious use of current best evidence in making decisions about the care of individual patients [346], it is crucial to ensure that QACs that have been tested *in vitro* have equivalent antimicrobial potential in *in vivo* settings before they are marketed commercially as “antimicrobial dental materials”. Moreover, clinically relevant *in-situ* models are required to validate the effects of novel antibacterial dental materials. Double-blind randomized control clinical trials remain the gold standard in evaluating treatment outcomes [347], [348]. In a case-controlled observational mini-study (ClinicalTrials.gov: registration number [NCT00299598](#)), ten volunteers wore removable acrylic appliances with two control resin composite specimens and two resin composite specimens incorporating 1 wt% QPEI nanoparticles for 4 h to develop intraoral biofilms [230]. The vitality of biofilms on QPEI-containing specimens was reduced by more than 50%, while those grown on the control resin composite discs were predominantly viable. No inflammation or allergic reaction developed after wearing of the appliances containing the experimental specimens. Recently, a double-blind, randomized clinical trial (RCT) was conducted to examine the *in vivo* antimicrobial efficacy of QAMS-containing orthodontic acrylic (ClinicalTrials.gov: registration number [NCT02525458](#)) [291]. The trial involved intraoral wearing of custom-made removable retainers by thirty-two human subjects to create 48-h multi-species plaque biofilms, using a split-mouth study design. Using confocal laser

scanning microscopy, it was found that incorporation of 5 wt% QAMS in a PMMA-based orthodontic acrylic resin enables killing of microorganisms in the plaque biofilms without harm to the oral mucosa or systemic health (Fig. 15).

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Fig. 15

Randomized clinical trial on the *in vivo* antimicrobial efficacy of quaternary ammonium methacryloxy siloxane (QAMS)-containing orthodontic acrylic. A. Schematic illustrating the relationship between a test disk and its fitting well within the retainer appliance. The disk surface to be examined was turned toward the palate but not in direct contact with the palate. Each well was protected from the oral cavity by orthodontic acrylic to prevent disturbance of the biofilm by the tongue. B. Each appliance contained 4 wells to house two 6-mm diameter retrievable control disks and 2 similar diameter retrievable experimental PMMA disks (containing 5 wt% QAMS). C. BacLight-stained confocal laser scanning microscopy merged image showing the presence of live (green) bacteria and fungal hyphae (open arrowhead) within the biovolume of a biofilm taken from a control disk of one subject. D. The corresponding BacLight-stained merged image showing the presence of dead (red) bacteria and fungal hyphae killed by the QAMS-containing acrylic from an experimental disk of the same subject. [291], Copyright 2016. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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It was previously perceived that polymerized “contact killing” antibacterial resin polymers exhibit antimicrobial activities only via direct contact of bacteria, without antibacterial effects against planktonic (free-floating) bacteria [349]. However, recent studies of QA-containing materials conducted with confocal laser scanning microscopy and three-dimensional reconstruction of biofilms stained with live-dead fluorescent stains [350], [351] indicate that those materials possess a reducing spectrum of biocidal activities from the substrate surface to the top of the biofilms [230], [288], [289], [352], [353]. That is, the presence of dead microorganisms is not limited to the bottom of the biofilms that is in contact with the antibacterial surface. This phenomenon may be explained by the ability of bacteria to undergo quorum-sensing signaling-induced programmed cell death in responses to environmental stresses [354], [355]. Activation of altruistic cell death mechanisms stimulates the release of pheromones such as the toxin component of toxin-antitoxin systems or bacteriocins (bacteriolytic molecules) [356], [357]. This coordinated sacrificial lifestyle in the biofilm community enables elimination of non-competent members of the community to sustain survival of the remaining members or for re-colonization. For *S. mutans* biofilms, a quorum-sensing pathway is present for inducing bacteriocin production in response to environmental stresses. The pathway responds to the intracellular accumulation of competence-stimulating peptide pheromone [358]. The occurrence of apoptosis-like programmed cell death in bacteria provides a rational explanation for the observation of three-dimensional killing by QACs within *in vivo* plaque biofilms that mimic the process of diffusional-killing by leached antimicrobial agents incorporated into polymers (Video 1 in Supplementary material; https://youtu.be/Iw5StFwE_FU).

7. Toxicological aspects of QACs

Although QAC-based biomedical materials have seen exciting advances, there are public concerns and even frustrations regarding their potential harm to the ecosystem and human health due to recent toxicological reports on QACs. Detection of QACs and their degradation products have been reported in diverse environments such as surface water, sediment and sludge-amended soil. The toxicity of QACs has received considerable attention because in addition to microorganisms, QACs are toxic to aquatic organisms, soil organisms, animals and humans [359]. Toxicity data from laboratory studies are essential for evaluation of the possible biological risks of QACs. Sandbacka et al. reported that QACs exhibited acute toxicity on fish cells, daphnia magna and even white fish [360]. Other studies reported that QACs exhibited higher toxicity in algae than fish and crustaceans [359], [361], [362], [363], due to their high affinity for negatively-charged algal cell walls. Sludge containing QACs also exhibit toxicity towards soil organisms such as earthworms [364]. Accumulation of QACs, together with other contaminants in soil over time, may change the soil environment and lead to potential bioaccumulation in soil organisms, resulting in potential biological risks [365]. Hence, the toxicological effects of QACs after their long-term exposure to the environment warrant further investigation [366].

Special attention should be paid to the toxicity of QACs towards animals and humans. The common QAC surfactant benzalkonium chloride causes toxicity and genotoxicity in *Daphnia magna* and *Ceriodaphnia dubia* models [367]. Using a eukaryotic cell model, genotoxicity has been observed for two QACs, benzalkonium chloride and dimethyldioctadecyl-ammonium bromide, at concentrations commonly found in waste water [368] and in commercially available nasal preparations [369]. These data suggest that direct contact with QA-containing detergents and pharmaceuticals that contain substantially higher QAC concentrations may cause potential DNA-damaging effects in humans. Different cell types have dissimilar susceptibility to the same QAC. An *in vitro* study conducted by Sokolova and coworkers reported that QACs exhibit selective toxic action among different cell lines [85]. Such a phenomenon may be explained by the different cell culture conditions and selective toxicity of QACs against lymphoblastoid cells compared with cells of epithelial origin. Melin et al. reported that QACs significantly reduced reproductive function in both male and female mice [370], [371]. Larsen et al. also found that inhalation of QAC aerosols results in acute airway irritation and inflammation in mice [372]. Theoretically, the QACs cannot be released from QA-based biomedical materials. Thus, a better understanding of the mechanisms of QAC-related toxicity should promote the innocuous use of these technologies.

The antimicrobial activity of QACs has generally been attributed to their ability to destroy cell membrane structure. To investigate the mechanisms responsible for the toxicity of QACs, Vieira's group compared the toxicity of QACs to cells cultured *in vitro* with their ability to kill pathogens [373]. The authors also evaluated the cytotoxicity of QACs toward several mammalian cell lines [374]. Unexpectedly, they found that cytotoxicity is intracellular in origin at low QAC concentrations; abnormality or damage of intracellular biochemical processes are involved in lieu of membrane disintegration or cell lysis. It has been shown that QACs at low concentrations induce oxidative stresses, trigger apoptotic signals and destroy cells by apoptosis [375], [376]. Similarly, DMAE-CB monomers cause overproduction of reactive oxygen species, cell cycle arrest, reduction in mitochondrial membrane potential and enhanced caspase-3 activity [61]. Mitochondria are believed to be a core component of the cell death machinery. Mitochondria act as sensors and amplifiers in determining the execution of cell death or apoptosis through regulation of cellular energy metabolism (mitochondrial respiration and oxidative phosphorylation) [377]. Mitochondrial dysfunction and perturbation of cellular energetic mechanisms are involved in QAC-induced toxicity [367], [378], [379], [380]. At very low concentrations, QACs inhibit mitochondrial respiration and oxidative phosphorylation. Apoptosis occurs when the cellular energy charge is reduced

by more than 50%. An *in vitro* study showed that QACs alter DNA structure and compromise vital cellular processes [381]. Recently, DMAE-CB monomers were found to induce intracellular oxidative stress, oxidative DNA damage and induction of intrinsic mitochondrial apoptosis [382]. Taking together, oxidative stress and oxidative damage are involved in QAC-induced cytotoxicity.

Based on the finding that oxidative stresses are involved in the cytotoxic effects of QACs, antioxidants such as N-acetyl cysteine (NAC) have been used for scavenging ROS and antioxidants to reduce the cytotoxicity of QACs [53], [60]. Modification of the molecular structures of QACs (e.g. preparation of pegylated-polymers) is an alternative strategy for combating their toxicity. It has been demonstrated that incorporation of poly(ethylene glycol) in QACs completely suppresses hemolysis of human red blood cells [383], [384]. Well-defined pegylated-polymers have been synthesized via RAFT polymerization [385]. By modifying the content of hydrophobic groups and molecular weights, the antimicrobial and hemolytic activities of amphiphilic polymethacrylate derivatives can be tailored. Compared with pegylated-polymers consisting of longer alkyl spacers, polymers with a shorter alkyl or hydroxyl group exhibit minimal hemolysis of red blood cells while retaining potent antimicrobial activities.

Quaternary ammonium-based nanomaterials may cause latent nanoparticle-related toxicity. Nano-sized particles have unique bio-distribution due to their highly different pharmacokinetic properties. Although existing drugs or materials are safe in the micrometer dimensions, unpredictable adverse and toxicological effects may emerge when they are formulated at the nanoscale. Nanoparticles are small enough to penetrate blood-brain barrier in the body, causing neurotoxicity [386]. Several approaches have been suggested and identified to achieve safe design of nanomaterials, based on the understanding of the bio-nanointerface [387] (Fig. 16). These approaches include: (1) changing the surface characteristics (such as size, surface charge, and dispersibility, particularly the effect of hydrophobicity) and the ability of nanoparticles to aggregate, (2) coating nanoparticles with protective shells, and (3) designing nanoparticles with different oxidative states.

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[Fig. 16](#)

Schematic representation of the interface between a nanoparticle and the lipid bilayer of a cell membrane. The nano-bio interface comprises the surface of the nanoparticle; the solid-liquid interface and the effects of the surrounding medium; and the interface's contact zone with biological substrates.

8. QACs and antimicrobial resistance

Antimicrobial resistance of pathogens is rapidly becoming the most serious concern in contemporary infection control [388]. A new post-antibiotic era has instigated since the discovery of untreatable strains of Enterobacteriaceae that are resistant to the carbapenem class of antibiotics, considered the “drug of last resort” for such infections [389]. Until recently, QACs were thought to be invulnerable to the development of antimicrobial resistance [390]. However, recently acquired data has shown this to be far from the truth. Therefore, comprehending the contributory conditions and underlying mechanisms that result in antimicrobial resistance of QACs are still required.

At concentrations above the minimum inhibitory concentration, it is well accepted that QACs interact with cell membranes of microorganisms, disrupt their membrane integrity and cause leakage of cellular contents [47]. However, QACs are biodegradable under aerobic conditions. As a result, microorganisms are dynamically exposed to QACs over a wide range of concentrations (i.e., non-inhibitory, sub-inhibitory, over-inhibitory concentrations). In general, environmental concentrations of QACs, including those that are found in sewage, biological wastewater treatment units, surface water and sediments, are well below minimum inhibitory concentration values. Thus, it is possible to prolong the contact between QACs and microorganisms at sub-inhibitory concentrations. Such environments with QACs at low chemical reactivity become selective and favorable for the survival of clones with lower susceptibility, ultimately leading to evolution and selection of QAC-resistant bacteria [391]. Many excellent reviews indicate that QAC-related antimicrobial resistance occurs at sub-inhibitory concentrations through both intrinsic and acquired resistance mechanisms [392], [393] (Fig. 17). Bacteria compensate for the oxidative stresses created by exposure to QACs at sub-inhibitory concentrations via SOS-response and induction of stress-response sigma factors rpoS inducing modification of cell membrane structure and composition, enhanced biofilm formation, hyper-expression of efflux pumps and acquisition of efflux pump genes through mobile recombination elements [394]. Mutations in QAC-resistant bacterial strains cause modifications in cell membrane, resulting in reduction in cell permeability and a more anionic and hydrophobic cell surface. Such modifications help reduce or restrict QAC-induced contact killing [395]. Exposure to QACs at sub-inhibitory concentrations may enhance biofilm formation [396], [397] through augmentation of certain gene expression, such as stress response sigma factor HrcA. The latter encodes a transcriptional regulator of the class I heat-shock response [398], and DnaK which encodes a class I heat-shock response chaperone protein [399]. The reduced expression of porins, which results in the changes of membrane protein composition, is also attributed to QAC resistance [400], [401]. Efflux pumps, which are generally chromosomally-encoded, help remove antimicrobial agents from the inside of bacterial cell [402]. Efflux-mediated QAC resistance may involve two mechanisms. First, QAC resistance is achieved by hyper-expression of efflux pumps or increase in their extrusion efficiency, including regulatory modifications or mutations of efflux determinants [403]. For instance, a study by Guo et al. showed that over-expression of efflux-related genes in a variant of *Salmonella typhimurium* increases their tolerance to benzalkonium chloride [404]. Another study by Buffet-Bataillon et al. demonstrated that clinical *E. coli* strains, variants of which are pathogenic, exhibit higher minimum inhibitory concentrations of QACs and ciprofloxacin. Their antibiotic resistance depends on the AcrAB-TolC efflux pump and its regulatory genes [405]. The second mechanism of QAC resistance is through acquisition of genes for specialized QAC efflux pumps through mobile recombination elements, such as plasmids, transposon or integrons. Acquisition of genetic elements, which can be collaterally transferred between bacteria of the same or different genera via quorum sensing, may result in co-resistance (two or more resistance mechanisms in one organism) or cross-resistance (one resistance mechanism that counteracts two or more antibacterial agents). The emergence of “small multidrug resistance” includes expressions of both multidrug efflux pumps [406] and QAC-specific efflux determinants [407]. Accordingly, antibiotic efflux mechanism is likely to be a potential therapeutic target to counteract antimicrobial resistance. There is limited *in vitro* evidence showing that efflux inhibitors such as verapamil and reserpine can block efflux pumps [408]. Further research is required to enhance the pharmacokinetics of these drugs and improve their biocompatibility to target QAC resistance by bacteria.

[Fig. 17](#)

Pathways and mechanisms of antimicrobial resistance in quaternary ammonium compounds (QACs). Bacteria compensate for exposure to QACs at sub-minimal inhibitory conditions via SOS-response and stress-response sigma factors rpoS-induction. These factors, in turn, induce modifications in bacteria cell physiology that result in the development QAC resistance. These modifications include induction of QAC biodegradation, enhanced biofilm formation, modifications in cell membrane via reduced expression of porins, acquisition of efflux genes such as plasmids, transposon or integrons, and over-expression of efflux pumps which helps remove antimicrobial agents from the inside of a bacterial cell with high extrusion efficiency. These modifications help reduce or restrict QAC-induced contact-killing. [394], Copyright 2009.

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Regarding QAC-based biomedical materials, there is an urgent need to evaluate whether the long-term use of such technologies results in reduced microbial susceptibility to the QAC and the emergence of QAC-resistant strains. A recent study investigated whether *S. mutans* and *E. faecalis* could develop resistance to the cationic biocides [409]. There were no changes in the minimum inhibitory concentration of MDPB to *S. mutans* and *E. faecalis* even after ten passages, suggesting that no antimicrobial resistance has been developed. Although experimental and clinical evidence on the causal link between QAC-based biomedical materials and antimicrobial resistance are rare, it is important to bear in mind that the use of QACs at sub-inhibitory concentrations helps develop QAC-resistant strains. Caution would be required in the use of QAC-based biomedical materials.

9. Concluding remarks and future perspectives

In addition to their broad-spectrum antimicrobial activity, biomaterials with QA functionalities possess potent and long-term biocidal efficacy with non-leachable active species, better biocompatibility and no adverse effects on the mechanical properties of the carrier materials. The advent of new technologies and biomaterials design, especially the emergence of nanotechnology, brings unique opportunities and hope to the formulation of novel biomedical materials that contain QA-functionalized fillers/nano-fillers, to address the difficulties and drawbacks of incorporating antibacterial agents that exist in their conventional dimensions into those materials. Nevertheless, there is an ever-growing need to develop antimicrobial materials aiming at multi-species pathogenic microbes, since many microorganisms including bacteria, fungi, protozoa, prions and viruses are involved in microbial infections. Over the past two decades, the advent of various polymerization techniques and post-modification strategies has made it possible to produce QA-based antimicrobial surfaces with an unprecedented level of control over composition, structure, and properties. Despite phenomenal advances in the variety of polymerization techniques, the ongoing progress in each of these arenas indicates that there is still plenty of room for future development.

There are also growing concerns regarding the biocompatibility QACs. Considerable evidence points to their potential toxicological side-effects which may hamper their safe and population-wide applications. Along with the efforts to enhance their antimicrobial properties, lower cytotoxicity also needs to be achieved to expand their clinical use. Currently, many research studies are engaged in investigating structure-bioactivity relationship of QA-based biomedical materials to balance their antimicrobial activity

and cytotoxicity. Although the exact and detailed mechanism is still largely unknown, prior reports suggest that oxidative stress plays an important role in their cytotoxicity. Experimental evidence suggests that the use of antioxidants helps reduce their cytotoxicity [53], [60], [410]. Some QACs may induce differential changes in expression of antioxidant enzymes such as superoxide dismutase, glutathione peroxidase, and catalase [382]. The expression of these antioxidant enzymes is under the control of nuclear factor E2-related factor 2 (Nrf2), a transcription factor that plays a key role in the intracellular anti-oxidative defense system to maintain redox homeostasis [411]. The aforementioned pathway also plays a protective role in nanomaterial-induced oxidative DNA damage and cell death [412], [413]. Accordingly, modulation of the Nrf2-mediated cellular defense response may help control QAC-induced cytotoxicity, an important issue that requires further investigation.

Sub-inhibitory concentrations of QACs may be involved in the evolution and selection of QAC-resistant bacteria through intrinsic or acquired resistance mechanisms. To date, there are no relevant studies that examine whether QA-based biomedical materials cause antimicrobial resistance. Nevertheless, one has to be careful with the use of QA-based biomedical materials. The biological environment within the human body is complex, and different fields of application require antimicrobial biomaterials with additional specific properties (e.g. remineralization or protein-repellent properties). Some research groups have incorporated additional components such as AgNPs [268], [269], [270], NO-releasing agents [173], [174], fluoride [414], NACPs [232], [233], [234], [235], [236], [237], [335] and 2-methacryloyloxyethyl phosphorylcholine [415], [416], [417] into QA-based biomedical materials to achieve augmented antimicrobial or multifunctional applications.

Biofilms are made up of a diverse community of interacting microorganisms with complex three-dimensional structures, including channels, micro-colonies and mushroom-like protrusions [418]. The properties of biofilms are distinct from those exhibited by planktonic species. Biofilms are more tolerant of adverse growth conditions such as antimicrobial agents, stress and host defense peptides. Thus, a better understanding of every aspect of the biofilm, especially with fluorescence microscopy coupled with functionalized nano-probes which allow *in-situ* investigations of the three-dimensional structure of biofilms, may help manage and eradicate biofilms. Recently, luminescent silica nanoparticles with QA functionality have been designed and synthesized [419]. Using confocal laser scanning microscopy, narrow diffusion paths of these luminescent nanoparticles were identified in *P. aeruginosa* biofilms. In addition, pH-sensitive luminescent silica nanoparticles have also been used to investigate pH microenvironment of biofilms of *E. coli* [420]. Future investigations with these functionalized nano-probes may render it possible to create a luminescent adhesive or resin composite with self-diagnostic capability using this technology. To expand the clinical use and commercialization of more QA-based biomedical materials, appropriately designed and well-structured multicenter clinical trials are critically needed to obtain reliable comparative data. Acquisition of these important information should help identify the most effective antibacterial solutions and the optimal circumstances for the use of these materials.

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Footnotes

Appendix A. Supplementary data

The following is Supplementary data to this article:

Video S1:

Representative three-dimensional confocal laser scanning microscopy (CLSM) video of biofilm grown on the surface of quaternary ammonium methacryloxy silane-containing acrylic resin (green channel: live microorganisms; red channel: dead microorganisms).

https://youtu.be/Iw5StFwE_FU.

[Click here to view.](#) (17M, flv)

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