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Review

Versatility of Silsesquioxane-Based Materials for Antimicrobial Coatings

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Abstract: The paper is dealing with the state of the art of antimicrobial nanostructured coatings with silsesquioxane content, along the last decade. The requirements of achieving efficient coatings, the chemical functionalization methods, the working mechanisms and a classification of different silsesquioxane-based materials are discussed to offer a complete overview of their most recent breakthrough for antimicrobial applications.

Keywords: antimicrobial; silsesquioxane; nanostructured coating; nanocomposite

1. Introduction

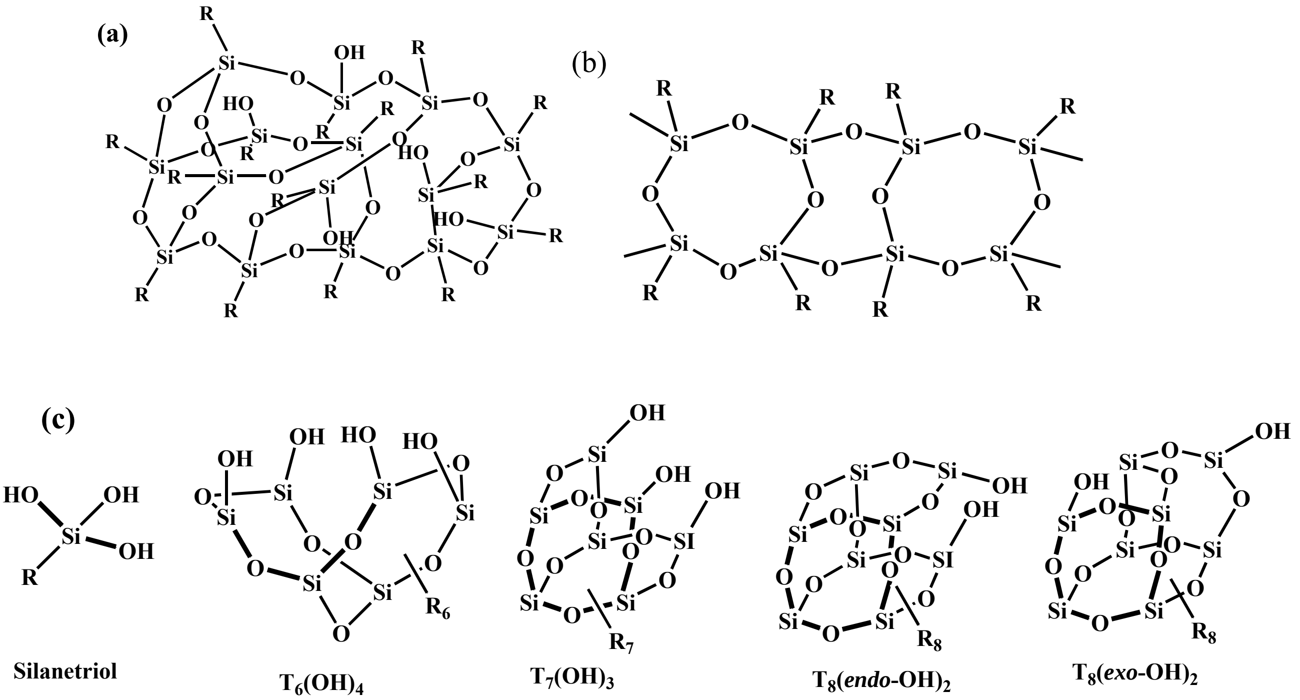
Infections caused by pathogenic microorganisms are common in many fields, especially in those related to water purification, food storage and packaging, medical devices, surgery equipments, dental restoration a.s.o, and are responsible for many deaths. In particular, these infections can be mostly found in hospitals that face a huge problem – that of microorganisms resistance to drug action. Given the ubiquitous nature of several types of germs (viruses, bacteria, fungi and protozoa) that can trigger the infection process, and the different peculiar situations in which these ones have direct contact with people (through breathing, touching, eating, drinking), it is quite surprising that the rate of diseases caused by them is not very high. For instance, *Pseudomonas aeruginosa* (gram-negative bacteria) and *Staphylococcus aureus* (gram-positive bacteria) are two of the most common opportunistic pathogens responsible for a wide range of infections. *Pseudomonas aeruginosa* is considered to be responsible for severe infections of people having medical problems and leading to mortality due to the significant damage of host tissues and resistance to antibiotics, while *Staphylococcus aureus*, that colonizes the throats and noses of more than 30 % of the total world population, is a common cause for food poisoning, respiratory diseases and skin infections. Moreover, the hospital strains of *Staphylococcus aureus* are more dangerous since they are usually resistant to the action of common antibiotics – the increased resistance of bacteria to antibiotics is a real major threat –, especially in case of polymicrobial infections (cumulative effect of two or more pathogens). As for fungal diseases, these ones are ranging from superficial infections (affecting the outer skin layers, nails and hair), oral candidiasis and vaginitis, to systemic or opportunistic infections that may have a devastating effect on people's health, especially under the influence of different types of *Aspergillus* fungus. One has to mention that humans are exposed, on a daily basis, to molds influence, either by breathing or touching, thus facing a serious threat.

Usually, these types of infections are treated by use of antimicrobial polymer coatings, a class of biocides presenting practical interest for protection against the microbes, used to reduce or stop the growth of organisms like bacteria and fungi, and representing an efficient alternative to the existing types of biocides and, in some cases, even to antibiotics. An ideal antimicrobial coating should manifest a prolonged activity, the ability to inhibit the growth of a broad spectrum of microorganisms, a high level of microbiocidal action, biocompatibility and low *in vivo* toxicity, should be cost-effective, reproducible and should have a minimum negative impact on the environment. Moreover, the choice of the antimicrobial polymer coating should take into account the possible damage induced by the presence of microorganisms, i.e., modification of surface properties, leaching of constituent additives, metabolites production mainly in the form of acids, enzymatic attack, impairment of polymer structural integrity through physical penetration, water accumulation, and pigments secretion [1].

This review presents the requirements for achieving efficient nanostructured coatings, as well as the main strategies employed in the synthesis of silsesquioxane-based materials designed for antimicrobial coatings.

2. Polyhedral oligomeric silsesquioxanes (POSSs)

Although antimicrobial coatings have been widely employed for several purposes, attempts to fabricate coatings possessing long-term antimicrobial properties are quite seldom, and this issue remains a big challenge. A possible solution to this problem has been offered by the use of silsesquioxane-based nanostructured materials. Silsesquioxanes are nanostructured materials (empirical chemical formula $(RSiO_{1.5})_n$) containing an inner core of inorganic silicon and oxygen atoms (nanostructured skeleton, cage or ladder structures) externally covered by polar or nonpolar organic groups (R). Based on their structural architecture, silsesquioxanes can be divided in two main categories – non-caged or incompletely condensed structures, containing silanol groups, and caged or completely condensed structures. The non-caged silsesquioxanes can be further classified into random (Figure 1, a), ladder (Fig. 1b), and partial-cage nanostructures (Fig. 1c) [2-4].



Silanetriol

$T_6(OH)_4$

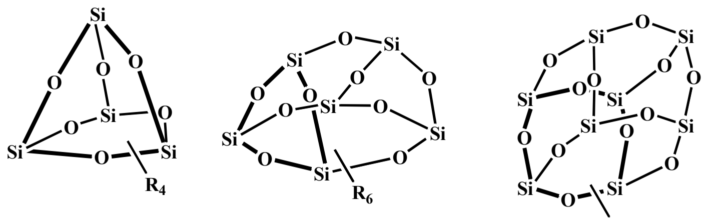
$T_7(OH)_3$

T_8(*endo*-OH)$_2$

T_8(*exo*-OH)$_2$

Figure 1. Different architectural structures of incompletely condensed silsesquioxanes: (a) random, (b) ladder and (c) partial-caged.

The caged silsesquioxanes are commonly known as polyhedral oligomeric silsesquioxanes (POSS) [5], and are illustrated as T_4, T_6, T_8, T_{10} and T_{12}, based on the number of silicon atoms present in the cubic structure (Figure 2).



T4

T6

T8

Figure 2. Schematic representation of cage-like silsesquioxanes (T_4, T_6 and T_8 structures).

Depending on R substituent nature (hydrogen, alkyl, alkylene or aryl arylene), POSS compounds are of different types – alkyls, olefins, alcohols, acids, amines, epoxies, thiols, fluoroalkyls, silanols, siloxides a.s.o. – and exhibit a high compatibility with polymers, acting as building blocks in the synthesis of organic-inorganic hybrid materials.

POSS molecules are introduced in polymer matrices by different methods [3]: (a) copolymerization of an organic monomer (styrene, methyl methacrylate) with POSS molecules having one or more comparable functional groups in their pendant arms, (b) blending of POSS molecules containing unreactive groups (alkyl groups) into the organic matrix, (c) grafting of a POSS unit with a single reactive function onto a pre-prepared polymer through the formation of covalent bonds, (d) direct crosslinking of POSS units, either directly through reaction of appropriate POSS derivatives with each other or by covalent binding to small organic molecules to form a three-dimensional network. As compared to other inorganic compounds, the POSS compounds used in the synthesis of hybrid nanocomposites provide unique characteristics such as a clearly defined structure and a small size of the silsesquioxane molecules (between 1-3 nm – the smallest silica particle [6]), a direct correlation between the number of organic groups and the number of corners, as well as a tunable reactivity of the organic functional groups, thus imparting the desired properties to the synthesized compounds. These organic-inorganic hybrid nanocomposites combine the advantages of organic materials (flexibility, processability and ductility) with the ones of the inorganic materials (thermal stability and rigidity).

The unique properties of POSS molecules lead to the development of novel hybrid nanocomposites with enhanced properties, including improved chemical, physical and mechanical characteristics. As follows, the incorporation of POSS molecules into polymers leads to lightweight nanocomposites characterized by an increased rigidity, strength, modulus and a reduction in viscosity, gas permeability, oxidation resistance of the material, heat discharge and flammability [7-9], a wide range of applications being reported – thermosetting [10-12] and thermoplastic polymers [13-15], polymer electrolytes [16-17], drug delivery systems [18-21], tissue engineering [22-25] a.s.o. Since POSS-based materials are highly biocompatible, their possible/expected biomedical applications have been intensely explored. As polymer coatings, functionalized POSS molecules are ideal for the production of unique hybrid nanocomposites, since such structural types are good carriers for biocide compounds, have highly branched structures containing ladder or cage fragments, and can be easily derivatized up to eight functional groups per molecule while maintaining a compact molecular size [26]. Moreover, the surface of the silsesquioxane-based coatings provides a nanoscopic topology that favors biomimetic function and cellular modulation [27] presenting interest from the interfacial assembly process perspective.

3. Antimicrobial applications of silsesquioxane-based materials

Although silsesquioxane-based materials themselves do not exercise any kind of antimicrobial action, their functionalization with biocidal agents is leading to efficient antimicrobial coatings with long-term resistance. An overview of the literature in the field evidences the existence of two main classes of silsesquioxane-based materials with antimicrobial properties, i.e., with quaternary ammonium groups and with metals.

3.1. Silsesquioxane-based materials with quaternary ammonium groups

Polymers containing quaternary ammonium groups (QAs) may be used as biocidal coatings that do not release biocide agents. They present a cationic character at all pH values, being composed of two different functional moieties, i.e., hydrophobic alkyl groups attached to a central positively charged nitrogen atom. QAs have been extensively used in commercial applications ranging from cosmetic conditioners and detergents to hospital disinfectants, antiseptics or herbicides. Due to their broad-spectrum antimicrobial activity, they present a number of advantages over other biocides – an effective action on a wide pH range, low vapor pressure, low human toxicity, as well as lack of unpleasant odors [28]. The dimensions of the hydrophobic tails are an important factor of influence, immobilized QAs possessing good antimicrobial properties due to their biocidal mechanism based on the so-called "contact killing". "Contact killing" can be easily understood – when in contact with the bacterial cell membrane, the long hydrophobic chains of QAs (high lipophilicity, surfactant character) show a high affinity for the bacterial cells (determined by the electrostatic attraction between the positively charged QAs and the negatively charged cell membrane) and the chains penetrate the cell membrane, a process followed by cytoplasmic membrane disruption and cell lysis [29-30]. The main steps of this action have been summarized by Kenawy *et al.* [31] as follows: (i) quaternary ammonium groups polymers adsorption on bacterial cell surface and (ii) diffusion through cell wall, (iii) adsorption onto cytoplasmic membrane, (iv) disruption of cytoplasmic membrane and (v) leakage of cytoplasmic membrane constituents, and finally (vi) cell death. Nevertheless, if the presence of a long hydrophobic chain is a prerequisite of a good biocidal activity, the formation of a shapeless mass due to the hydrophobic attraction between QAs units is also desirable.

As for the silsesquioxane-based materials functionalized with QAs components, the relatively reduced size of POSS molecules allows a charge density similar to that of dendrimers, thus enhancing the antimicrobial efficiency. Last years, several papers explored the antimicrobial properties of different silsesquioxane-based compounds quaternized with QAs units. One of the first papers, dealing with the incorporation of QAs units into POSS structures, described the synthesis of a dimethylamino-functionalized POSS quaternized (40 % quaternization degree) with 1-iodo-octane (Figure 3) and evidenced the good antimicrobial activity toward both gram-negative (*Escherichia coli*) and gram-positive (*Staphylococcus aureus*) bacteria, activity depending on alkyl chain length and charge density [32]. The experimental data evidenced a log 7 microbial growth reduction for both studied microorganisms after a 2 hours exposure time.

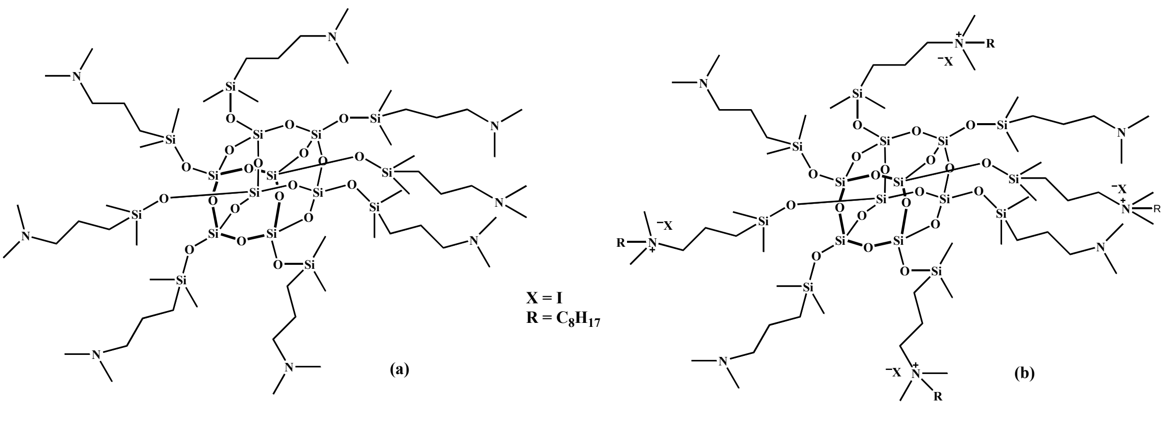


Figure 3. Schematic representation of (a) dimethylamino-functionalized POSS; (b) Q-POSS idealized structure.

Chojnowski *et al.* published an extensive work on the bacteriocidal activity of several oligo- and polysilsesquioxanes with ammonium salts of variable quaternization degrees (Figure 4) [33]. Two types of oligosilsesquioxanes, i.e., octa(3-chloropropylsilsesquioxane) and poly(3-chloropropylsilsesquioxane) were reacted with dimethyl-n-octylamine, the best antimicrobial activity, i.e., growth inhibiting of *Enterococcus hirae* and *Staphylococcus aureus* (gram-positive bacteria) as well as *Escherichia coli* and *Pseudomonas aeruginosa* (gram-negative bacteria) being attained for the compounds characterized by a 50 % conversion degree. The authors highlighted their potential use as bacteriocidal additives to other materials. The oligomers almost fully substituted with the ionic QAs units proved to be very active against gram-positive bacteria only in suspension, manifesting a lower activity in solution. It was suggested that the presence of too many ionic groups (bulky units) limits the conformational freedom of the isolated molecules in solution, thus weakening their interaction with bacterium cell wall. Polysilsesquioxanes containing secondary n-amylammonium salt groups were obtained through the reaction of n-alkyl bromides with low molecular weight poly(3-aminopropylsilsesquioxane) or by amination of n-alkyl bromide with 3-aminopropyltriethoxysilane, followed by hydrolysis and polycondensation. The last ones showed good or moderate bacteriocidal activity against gram-positive bacteria and were able to kill gram-negative bacteria only when used in high concentrations. As expected, a comparison of different ammonium salts polysilsesquioxanes evidenced the importance of the hydrophobic moiety of the ammonium group in killing bacteria.

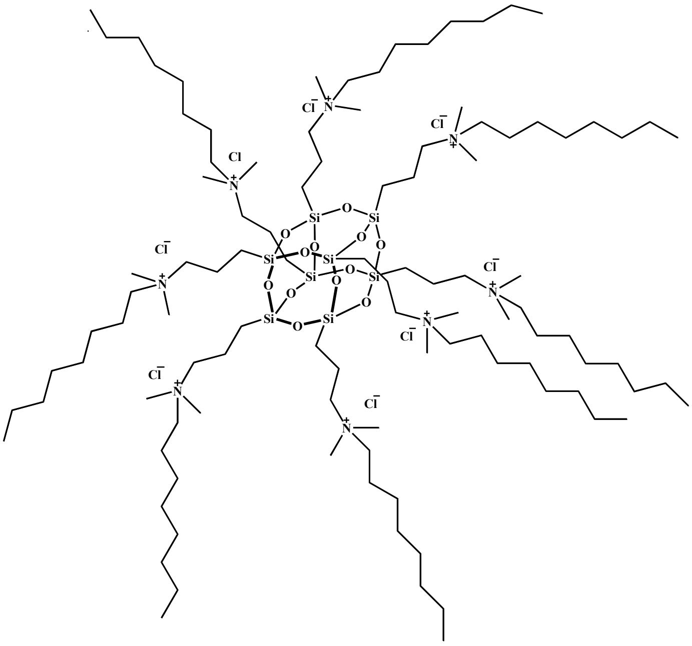


Figure 4. Schematic representation of a polysilsesquioxane containing secondary n-amylammonium salt.

Literature reports on the synthesis and aqueous solution antimicrobial properties toward *Escherichia coli* and *Staphylococcus aureus* of an array of quaternary ammonium-functionalized POSS compounds (Q-POSS) [34]. The synthesis involved the hydrosilylation of an octasilane POSS with allyldimethylamine, yielding a functionalized POSS containing eight tertiary amino groups, the quaternization reaction being performed with several iodoalkanes of varying chain lengths. The antimicrobial activity of Q-POSS compounds in aqueous solution was found to be strongly dependent on their composition. Due to the aggregation phenomenon that inhibited the diffusion through bacterial cells walls, lower quaternization extents and presence of longer alkyl chains determined the highest antimicrobial activity in solution, while for the highest quaternization extents the maximum antimicrobial activity was achieved for a twelve carbon atoms alkyl chain. To establish the utility of Q-POSS as antimicrobial additive for polysiloxane coatings, several Q-POSS compounds with different lengths and extent of quaternization were incorporated in two different moisture-curable polysiloxane coatings, a significant dependence of antimicrobial properties on Q-POSS composition being evidenced. The Q-POSS-based coatings possessing the lowest quaternization extent (~ 40 mol %) displayed a good antimicrobial activity, whereas no antimicrobial activity was exhibited by analogous coatings possessing the highest quaternization extent (~ 100 mol %). Q-POSSs were present at coating surface (nanoscale surface roughness) in the coatings of low quaternization, while in the case of the ones of high quaternization a significant aggregation of Q-POSS molecules was evidenced. This aggregation was due to both intermolecular ionic and intermolecular van der Waals interactions that inhibit the diffusion and interaction of Q-POSS molecules with bacterial cells.

To prepare potentially antimicrobial POSS aditives for polysiloxane coatings, a series of quaternization reactions of several octasilane Q-POSS compounds with different alkyl chain lengths (from $-C_{12}H_{25}$ to $-C_{18}H_{37}$), functionalized with QAs units through various counter ions, i.e., chlorine, iodine, bromine have been carried out [35]. Octasilane POSS was first functionalized with dimethylamino groups by hydrosilylation with allyldimethylamine, and the partial quaternization (~ 40 mol %) of the resulting tertiary amino-functional POSS was then achieved by using an alkyl halide (Figure 5). Both alkyl chain length and counter ion were found to affect Q-POSS antimicrobial properties, the highest antimicrobial efficiency against *Escherichia coli* and *Staphylococcus aureus* being proved by Q-POSS with C12 alkyl chain length and chlorine counter ion. The structure of this compound allows the most appropriate balance between the lipophilicity and diffusivity that induce both effective binding to the outer surface of bacterium cell structure and diffusivity through the cell wall to cell's interior.

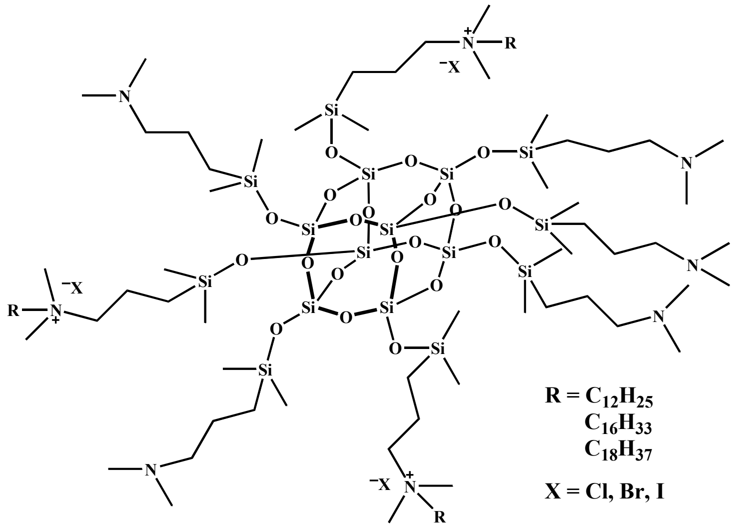
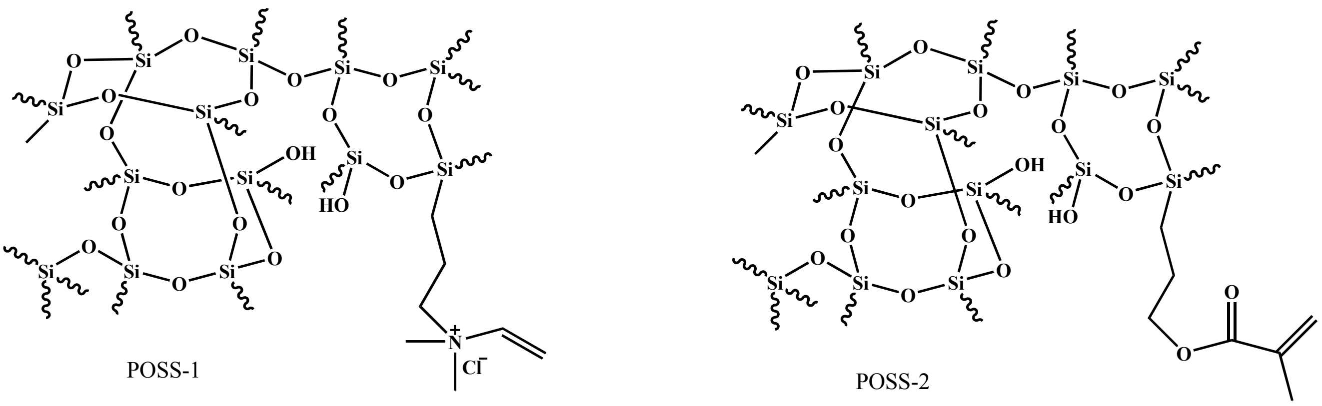


Figure 5. Schematic representation of octasilane Q-POSS compounds.

As for the efficiency of Q-POSSs incorporated into a moisture-curable polysiloxane coating, i.e., a blend solution containing silanol-terminated polydimethylsiloxane, methylacetoxysilane and a catalyst, the experimental data showed that both Q-POSS and polysiloxane matrix compositions affect the antimicrobial properties, all coatings being more efficient against *Staphylococcus aureus*, followed by *Candida albicans* fungus and *Escherichia coli*. The data proved that the interaction between the polysiloxane matrix and the Q-POSS alkyl chains is the main factor influencing the overall antimicrobial activity, while the antimicrobial activity of moisture-cured polysiloxane coatings is significantly influenced by the relative concentration of Q-POSS at coatings surface.

The first report on the use of hierarchical assemblies with silsesquioxane and quaternary ammonium units intended for antimicrobial monumental stone coating was published by Simionescu *et al.* [36]. A controlled, evaporative self-organization of two silsesquioxane-based polymer blend solutions, i.e., one containing methacrylate units and the other having quaternary ammonium units, in different molar ratios, yielded hierarchical structures comprising nanofibrillar micelles confined within semi-cylindrical shells. The silsesquioxane-based hybrid nanocomposites with quaternary ammonium units were obtained through quaternization of N-allyldimethylamine with (3-cloropropyl)trimethoxysilane followed by subsequent hydrolysis and polycondensation of the resulting compound (POSS-1), while the synthesis for the obtaining of the methacrylate units compound (POSS-2) involved a sol-gel reaction of a silane precursor, i.e, (trimethoxysilyl)propyl methacrylate, in the presence of POSS-1, both reactions being performed in acidic conditions. The two silsesquioxane-based hybrid nanocomposites were blended in different molar ratios, i.e., 1:2 (POSS-Q1) and 1:4 (POSS-Q2), respectively (Figure 6).



POSS-1

POSS-2

Figure 6. Presumed structure of silsesquioxane-based hybrid nanocomposites (POSS-1, POSS-2).

The formation of hierarchical assemblies possessing two different length scales – nanofibrillar micelles and semi-cylindrical shells – was ascribed to the presence of multiple intermolecular ionic interactions, intermolecular Van der Waals forces and hydrophobic interactions acting among the constituent molecules. Both silsesquioxane-based polymer blend coatings were more effective against *Staphylococcus aureus*, followed by *Candida albicans* fungus, while no action was registered against *Escherichia coli*. POSS-Q2 proved a higher antimicrobial activity as compared to POSS-Q1, although both compounds contained the same charge density of QAs units. The more efficient antimicrobial activity of POSS-Q2 was ascribed to its topographical features, i.e., a disconnected focal adhesion on the nanofibrilar pattern (nanofibers aggregated in bundles of several parallel nanofibers) inducing a reduced bacteria extension. POSS-Q1 nanofibers showed a local orientation ordering and collective bending, at a certain point, the nanofibrils becoming interconnected and thus leading to an increased number of contact points with bacteria cells. Moreover, different relative diameters of the nanofibrillar micelles were registered (nanofibers aggregated in bundless between 0.2 – 1.4 µm for POSS-Q1 and 116.6 – 641.1 nm for POSS-Q2, respectively), the bacterial adhesion forces being enhanced with increasing the nanofiber diameters.

One of the major problems for fouling is the undesirable attachment and growth of macro- and microorganisms on surfaces immersed in seawater. To address a solution to this problem, Liu et *al.* published a paper on the incorporation of QAs units to impart additional antibiofouling properties to polydimethylsiloxane (PDMS) materials that already proved to be excellent fouling-release coatings in marine environment (Figure 7) [37].

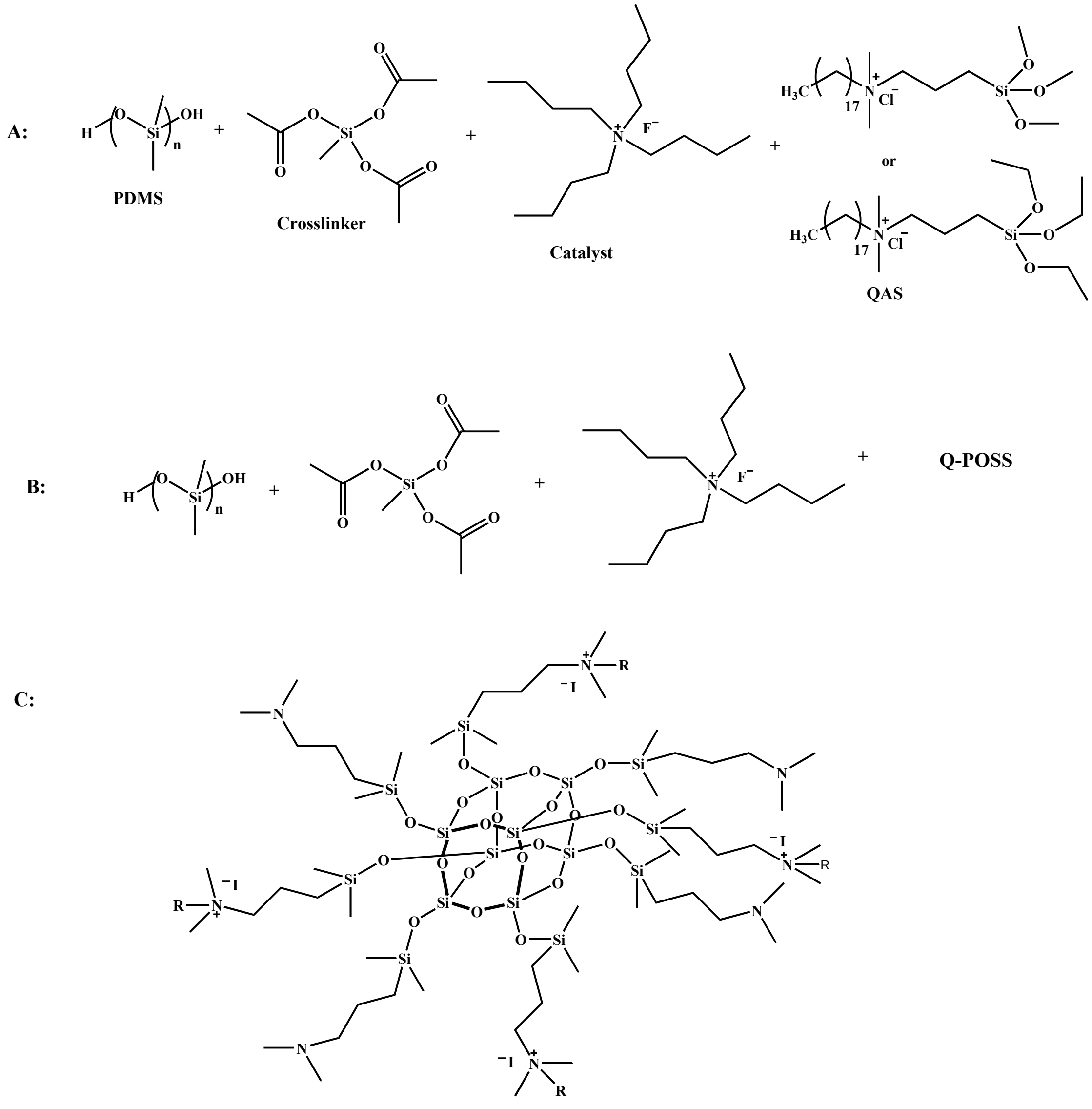


Figure 7. Chemical structures of QAs incorporated in PDMS systems: (A) QAs-tethered system, (B) Q-POSS incorporated in PDMS system, (C) Q-POSS structure.

More specifically, a series of PDMS coatings possessing either QAs units containing a single ammonium salt group/molecule or Q-POSS structures were synthesized, a relation between the interfacial surface structures and their antifouling properties being established. A direct corellation between the extent of quaternization, the nature of the QAs functional groups, and the molecular weight of PDMS component was found to be of main influence on surface properties. A lower extent of Q-POSS quaternization and use of ethoxy functional QAs groups facilitated the extension of the alkyl chains away from the nitrogen atoms, thus favouring the neutralization of marine microorganisms upon contact. The adsorption of bacteria as a result of the electrostatic attraction between the negatively charged microorganisms and the positively charged QAs nitrogen atoms on the surface was observed first, this process being followed by the disruption of cell membanes due to the penetration of QAs long alkyl chains.

3.2. Metal-containing silsesquioxane-based materials

The use of silver as antimicrobial agent has a history of at least 2000 years, silver vessels for water storage being well-known and widespread in ancient Rome, Greece and Egypt. Although the first mention on the medical use of silver is dated back to around 750 AD, its germicide action was first proven only in 1869 [38]. A detailed study dealing with the antimicrobial properties of silver was published by Berger et *al*. [39], who evidenced the upper (4 μg/mL Ag^+) and lower (1.25 μg/mL Ag^+) limits between which silver ions exhibited antibacterial efficiency against sixteen bacterial species. Moreover, the use of 4 μg/mL Ag^+ concentration did not induce a destablishing effect to mouse bone marrow cells. Although the field of antimicrobial coatings is constantly expanding, silver compounds remain among the most attractive antimicrobial agents due to their peculiar properties – broad antimicrobial efficiency against both gram-positive and gram-negative bacteria, as well as the presence of multiple cellular targets able to reduce the resistance development in bacteria [40]. At the same time, inspite its long use in antimicrobial applications, silver antimicrobial mechanism is not yet fully understood. Among the accepted mechanisms one can mention amino acid or DNA – silver interaction, the generation of reactive oxidative species, and cell membrane damage. The cytotoxicity of heavy metal ions (Ag^+, Cu^{2+}, Hg^{2+} a.s.o.) is due to the interaction between these ones and the thiol groups in proteins or enzymes, process which might affect the biological functions [41-42]. Due to the preferential interaction with DNA bases, silver ions can induce significant gene mutation and subsequent death of microbes [43-44]. A general consensus exists on the action and effect of silver ions, i.e., it is considered that their binding to bacteria intracellular proteins and nucleic acids affects cell viability; however, the detailed mechanism is not fully elucidated. Several hypotheses have been formulated to explain silver penetration and accumulation into cell membrane, i.e., silver accumulation due to the existence of electrostatic attraction between positively charged silver nanoparticles and negatively charged bacterial surfaces [45], thiol-silver interaction on cell surface [46], and generation of pits and holes in cell membrane and release of lipopolysaccharides from cell membrane [47-48]. As opposite to ionic or bulk metallic forms, the antimicrobial efficiency of silver nanoparticles is based on the catalytic generation of reactive oxidative species from dissolved oxygen in solution [49]. Besides silver nanoparticles, various types of silver nanostructures were reported to present good antimicrobial activity – silver nanofibers [50-51], silver-coated fibers [52], silver nanoflakes [53], silver-titania nanocomposites [54-55], crosslinked micelles and silver nanoparticle in the cores [56].

Different types of nanofibrous webs based on silver-containing thermoplastic hydrogels were obtained starting from multiblock poly(ethylene gylcol) – POSS polyurethanes (Figure 8) [57]. The hydrogels were co-dissolved with silver nitrate to yield durable hydrogel webs with efficient antimicrobial properties. The thermodynamic incompatibility between ethylene oxide and POSS units determined microphase separation phenomena, leading to a physical crosslinking and finally to the formation of hybrid hydrogels in swollen state. Upon immersion in water, the nanofibrous structures slightly shrink rather than expand, this unusual behavior being correlated to the orientation relaxation upon swelling. The lack of swelling could recommend such structures for wound dressing applications, this property minimizing the undesirable lateral wicking. The as-prepared nanofibrous webs were able to supress the formation of *Escherichia coli* biofilm for a 14 days extended period, this being atributed to their compact internal structure controlling the elution rate and availability of silver ions at the hydrogel surface, thus suggesting promising applications.

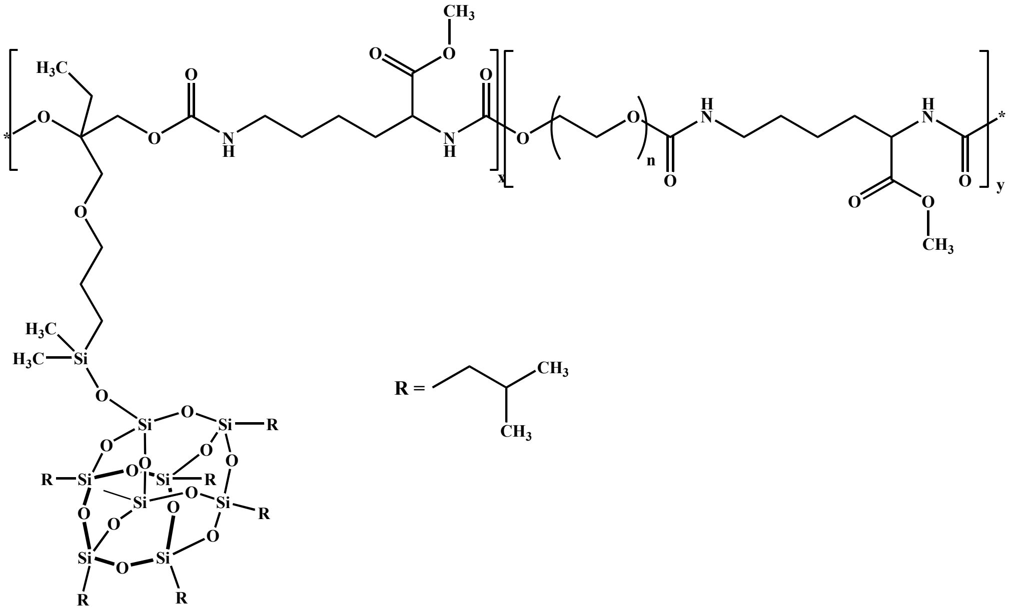


Figure 8. Schematic representation of PEG-based multiple block thermoplastic polyurethane incorporated into POSS macromer.

Two bilayered scaffolds were developed, one inner biodegradable layer based on a silsesquioxane-based hybrid nanocomposite incorporated inside a poly(caprolactone-urea)urethane (POSS-PCU) containing a poly(hexamethylenecarbonate) soft segment, (Figure 9, a) and one non-degradable outer layer based on a silsesquioxane hybrid nanocomposite incorporated inside a poly(caprolactone-urea)urethane (POSS-PCL) compound (Figure 9, b) [58]. The inner biodegradable POSS-PCU layer was designed to degrade following cell grow and maturation inside scaffold, while the removable and non-degradable outer layer scaffold was obtained through POSS-PCL impregnation with silver in order to induce antibacterial and antiinflamatory properties. The bilayered POSS-PCU/POSS-PCL scaffold exhibited a microporous topography, with a well defined distribution of pores and superior mechanical properties as compared to a commercially available scaffold. The integration of POSS nanotopographical features allowed a superior cellularization with adipose tissue-derived stem cells (ADSCs) having the diameter ranging from 50 to 100 nm, the cellular proliferation being achieved in a matter of days, leading to a rapid neurovascularization and dermis regeneration. The simple and inexpensive method of fabrication, as well as the good mechanical and added antibacterial and antiinflamatory properties recommend such bilayered scaffolds for wound healing applications.

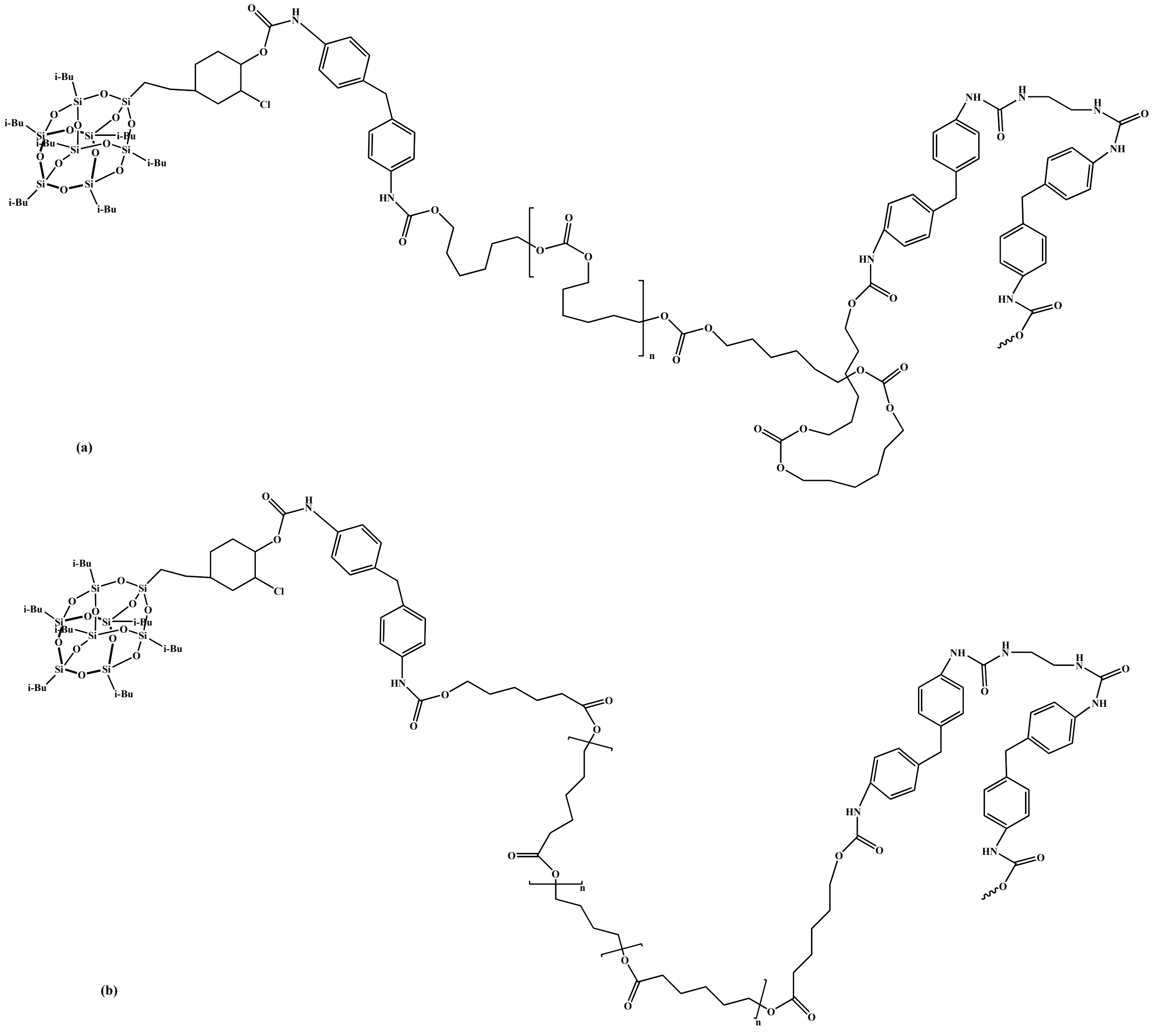


Figure 9. Schematic representation of (a) non-biodegradable POSS-PCU, (b) biodegradable POSS-PCL.

A first study on the use of silver nanoparticles in antibacterial coatings for monumental stones was published by Aflori *et al.* [55]. The sol–gel reaction of 3-(trimethoxysilyl)propylmethacrylate precursor or a combination between this one and titanium isopropoxide, in the presence of a primary amine surfactant and silver nitrate (silica : silver nitrate precursors = 1 : 0.1 molar ratio), yielded new types of silsesquioxane-based hybrid nanocomposites with methacrylate units, containing either only silver nanoparticles (POSS-Ag) or a combination of titania and silver nanoparticles (POSS-AgTi) intended for antibacterial coatings for building stones conservation (Figure 10). The self-assembling structures (semi-cylindrical shells) with homogeneous distribution of metalic nanoparticles have been ascribed to the formation of supramolecular assemblies and to a combination of silsesquioxane ladder and cage-like fragments. Both synthesized compounds showed high antibacterial/antifungal efficiency against *Escherichia coli* (gram-negative bacteria) and *Candida albicans* fungus, better results being obtained in POSS-AgTi case. The measurements being perfomed in the absence of UV light, the higher antibacterial/antifungal activity of the compounds was attributed to the action of solely silver nanoparticles or/and to the photocatalytic titania activity (titania doping with silver) in the visible domain.

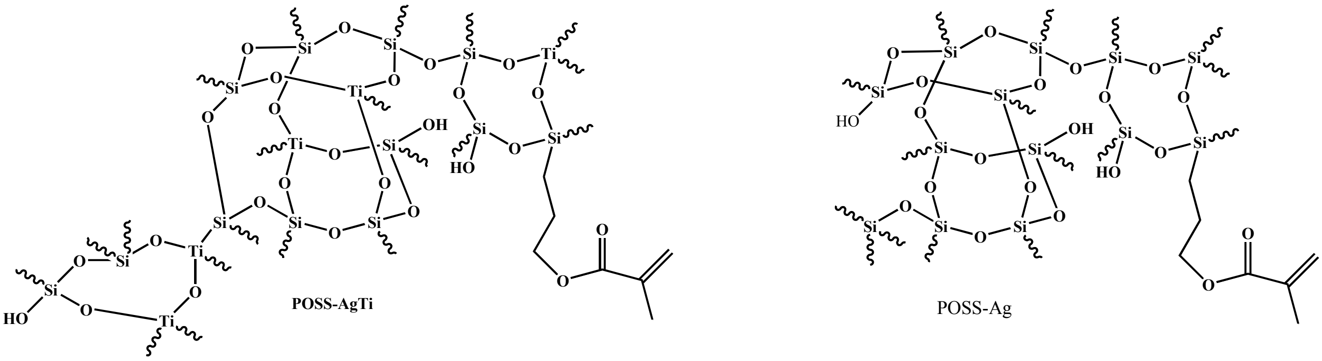


Figure 10. Presumed structure of silsesquioxane-based hybrid nanocomposites (POSS-Ag, POSS-AgTi).

A comparative study on the disinfection performance of ceramic water filters impregnated with either silver nanoparticles or poly(trihydroxysilyl)propyldimethyloctadecyl ammonium chloride (TPA), a silsesquioxane-based polymer with QAs units was published by Zhanga *et al.* [59]. The study describes some alternative solutions for finding disinfectant compounds less expensive than silver, the price of TPA being five times lower than silver's. At lower concentrations TPA exhibited higher antimicrobial efficiency against *Escherichia coli* (54 % percentage inhibition, 0.34 bacterial log reduction value (LRV)) than silver nanoparticles (30 % percentage inhibition, 5.8 LRV), while at higher concentrations both agents proved similar antimicrobial properties (TPA: 100 % percentage inhibition, 10.8 LRV; silver nanoparticles: 99 % percentage inhibition, 5.8 LRV). The antimicrobial mechanism of silver nanoparticles was ascribed to bacteria damage by pitting on cell membrane and cell lysis due to release of silver ions, while the TPA toxicity was attributed to the interaction between the positively charged quaternary amine groups and *Escherichia coli* membrane. However, further studies on TPA long-term toxicology are necessary to evaluate its use in ceramic water filters.

A new system composed of a mixture of ionic silsesquioxane and silver nanoparticles has been proposed as an efficient antibacterial agent [60]. Spherical nanoparticles were synthesized in the presence of a charged silsesquioxane with QAs units and 1,4-diazoniabicyclo[2.2.2]octane nitrate stabilizer. The zeta-potential measurements indicated that silver nanoparticles are stable and have a positively charged surface, the new system showing long term (more than one year) stability. *In vitro* and *in vivo* antibacterial tests performed against *Pseudomonas aeruginosa* (gram-negative bacteria), *Escherichia coli* (gram-negative bacteria) and *Staphylococcus aureus* (gram-positive bacteria) evidenced the minimum silver nanoparticles concentrations required for the complete inhibition of bacteria, i.e., 0.60, 1.1 and 2.0 μg mL^{-1}, respectively, while the cytotoxicity measurements showed that at the studied concentrations silver nanoparticles were safe for mammalian cells.

Another metal proposed as antibacterial agent is cooper, its different forms being used in antiquity to treat wounds, to maintain hygiene, and to sterilize the drinking water [61]. The soluble forms of copper compounds have proved to be efficient against a large number of bacteria, algae, fungi and viruses while maintaining a low toxicity (i.e., being relatively safe) for humans [62]. Copper's "killing effect" acts through the generation of reactive hydroxyl radicals that induce damage to cell bacteria – protein oxidations, DNA and RNA cleavage, lipid peroxidation [63-64]. Although copper bioactivity – as ion or in complex forms – is well-known, few papers were dealing with copper-containing nanomaterials [65-67].

A significant challenge for copper-based coatings was the inclusion of copper nanoparticles inside a solid structure with antibacterial properties while preventing copper leaching into the environment. Silsesquioxanes proved to effectively bind copper nanoparticles to the substrate without allowing its release. The best results were obtained with silsesquioxanes bearing multiple functionalities (up to three), the specific functional groups being selected to provide specific requirements, i.e., copper and hydroxyl binding affinity and non-reactivity *vs.* silsesquioxane linking capacity [68]. The multifunctional silsesquioxanes were designed to act as coupling agents to both copper and natural textiles such as cotton, a solvent-based liquid coating system being developed. One may expect that these types of nanomaterials will reduce hospital infections by 10 % – a really impressive value.

A patent application [69] reported the obtaining of transparent cover glasses with antimicrobial properties covered with polysiloxanes/silsesquioxanes protective coatings containing copper or Cu_2O nanoparticles. The protective coatings proved efficiency in protecting the surface from oxidation process while maintaining a good long-term antimicrobial activity (the tests were performed on *Escherichia coli* at a 10^5 bacteria/mL concentration). These transparent cover glasses with antimicrobial properties may be used in any "touch" application, such as computers and cell phones.

4. Conclusions

The present article discusses two main strategies to design silsesquioxane-based antimicrobial materials active against a wide-range of microorganisms, i.e., incorporation of quaternary ammonium units and incorporation of metals.

Their antimicrobial activity may be induced through chemical modification or though introduction of inorganic antimicrobial agents. Recent research has been focused on POSS structures with quaternary ammonium units, although such type of systems are not always the best solutions to assure an efficient antimicrobial coating against a wide-range of microorganisms. Since POSS materials are resistant to degradation, biocompatible, safe, compliant, anti-thrombogenic, and allow neo-endothelialization, it is expected that new lines of research will be developed in the field of antimicrobial coatings. Reduction of cytotoxicity while maintaining or even enhancing the antimicrobial activity will stand among the efforts to be made in the future.

Conflicts of Interest

The authors declare no conflict of interest.

References and Notes

1. Flemming, H.-C. Relevance of biofilms for the biodeterioration of surfaces of polymeric materials. *Polym. Degrad. Stabil.* **1998**, *59*, 309–315.
2. Kuo, S.W.; Chang, F.C. POSS related polymer nanocomposites. *Prog. Polym. Sci.* **2011**, *36*, 1649–1696.
3. Cordes, D.B.; Lickiss, P.D.; Rataboul, F. Recent developments in the chemistry of cubic polyhedral oligosilsesquioxanes. *Chem. Rev.* **2010**, *110*, 2081–2173.
4. Wu, J.; Mather, P.T. POSS polymers: Physical properties and biomaterials applications. *Polym. Rev.* **2009**, *49*, 25–63.
5. Li, G.Z.; Yamamoto, T.; Nozaki, K.; Hikosaka, M. Crystallization of ladderlike polyphenylsilsesquioxane (PPSQ)/isotactic polystyrene (i-PS) blends. *Polymer* **2001**, *42(20)*, 8435–8441.
6. Li, G.Z.; Wang, L.C.; Toghiani, H.; Daulton, T.L.; Koyama, K.; Pittman, C.U. Viscoelastic and mechanical properties of epoxy/multifunctional polyhedral oligomeric silsesquioxane nanocomposites and epoxy/ladderlike polyphenylsilsesquioxane blends. *Macromolecules* **2001**, *34(25)*, 8686–8693.
7. Camargo, P.H.C.; Satyanarayana, K.G.; Wypych, F. Nanocomposites: Synthesis, structure, properties and new application opportunities. *Mater. Res.* **2009**, *12*, 1–39.
8. Chan, E.R.; Striolo, A.; McCabe, C.; Cummings, P.T.; Glotzer, S.C. Coarse-grained force field for simulating polymer-tethered silsesquioxane self-assembly in solution. *J. Chem. Phys.* **2007**, *127*, 114102.
9. Zhou, Z.; Cui, L.; Zhang, Y.; Zhang, Y.; Yin, N. Preparation and properties of POSS grafted polypropylene by reactive blending. *Eur. Polym. J.* **2008**, *44*, 3057–3066.
10. Chen, Q.; Xu, R.; Zhang, J.; Yu, D. Polyhedral oligomeric silsesquioxane (POSS) nanoscale reinforcement of thermosetting resin from benzoxazine and bisoxazoline. *Macromol. Rapid Comm.* **2005**, *26(23)*, 1878–1882.
11. Zeng, K.; Zheng, S. Nanostructures and surface dewettability of epoxy thermosets containing hepta(3,3,3-trifluoropropyl) polyhedral oligomeric silsesquioxane-capped poly(ethylene oxide). *J. Phys. Chem. B* **2007**, *111*, 13919–13928.
12. Pinson, D.M.; Yandek, G.R.; Haddad, T.S.; Horstman, E.M.; Mabry, J.M. Thermosetting poly(imide silsesquioxane)s featuring reduced moisture affinity and improved processability. *Macromolecules* **2013**, *46(18)*, 7363–7377.
13. Bourbigot, S.; Turf, T.; Bellayer, S.; Duquesne, S. Polyhedral oligomeric silsesquioxane as flame retardant for thermoplastic polyurethane. *Polym. Degrad. Stabil.* **2009**, *94(8)*, 1230–1237.
14. Seurer, B.; Coughlin, E.B. Ethylene–propylene–silsesquioxane thermoplastic elastomers. *Macromol. Chem. Phys.* **2008**, *209*, 1198–1209.
15. Gu, X.; Wu, J.; Mather, P.T. Polyhedral oligomeric silsesquioxane (POSS) suppresses enzymatic degradation of PCL-based polyurethanes. *Biomacromolecules* **2011**, *12(8)*, 3066–3077.

16. Chinnam, P. R.; Wunder, S.L. Polyoctahedral silsesquioxane-nanoparticle electrolytes for lithium batteries: POSS-lithium salts and POSS-PEGs. *Chem. Mater.* **2011**, *23(23)*, 5111–5121.
17. Zhang, F.; Tu, Z.; Yu, J.; Li, H.; Huang, C.; Zhang, H. Impregnation of imidazole functionalized polyhedral oligomeric silsesquioxane in polymer electrolyte membrane for elevated temperature fuel cells. *RSC Adv.* **2013**, *3*, 5438–5446.
18. Nair, B.P.; Vaikkath, D.; Nair, P.D. Polyhedral oligomeric silsesquioxane-F68 hybrid vesicles for folate receptor targeted anti-cancer drug delivery. *Langmuir* **2014**, *30(1)*, 340–347.
19. Ghanbari, H; Cousins, B.G.; Seifalian, A.M. A nanocage for nanomedicine: polyhedral oligomeric silsesquioxane (POSS). *Macromol. Rapid Comm.* **2011**, *32(14),* 1032 –1046.
20. Kaneshiro, T.L.; Lu, Z.R. Targeted intracellular codelivery of chemotherapeutics and nucleic acid with a well-defined dendrimer-based nanoglobular carrier. *Biomaterials* **2009**, *30(29)*, 5660–5666.
21. Guo, Q.; Knight, P.T.; Mather, P.T. Tailored drug release from biodegradable stent coatings based on hybrid polyurethanes. *J. Controlled Release* **2009**, *137,* 224–233.
22. Guo, Y.; Li, M.Y.; Mylonakis, A.; Han, J.J.; MacDiarmid, A.G.; Chen, X.S.; Lelkes, P.I.; Wei, Y. Electroactive oligoaniline-containing self-assembled monolayers for tissue engineering applications. *Biomacromolecules* **2007**, *8,* 3025–3034.
23. Raghunath, J.; Zhang, H.; Edirisinghe, M.J.; Darbyshire, A.; Butler, P.E.; Seifalian, A.M. A new biodegradable nanocomposite based on polyhedral oligomeric silsesquioxane nanocages: cytocompatibility and investigation into electrohydrodynamic jet fabrication techniques for tissue-engineered scaffolds. *Biotechnol. Appl. Biochem.* **2009**, *52*, 1–8.
24. Kannan, R.Y.; Salacinski, H.J.; Ghanavi, J.-E.; Narula, A.; Odlyha, M.; Peirovi, H.; Butler, P.E.; Seifalian, A.M. Silsesquioxane nanocomposites as tissue implants biomaterials and tissue engineering center. *Plastic and reconstructive surgery* **2007**, *119(6)*, 1653–1662.
25. Nakamura, J.; Kasuga, T. Enhancement of crystalline plane orientation in silsesquioxane-containing vaterite particles towards tuning of calcium ion release. *J. Mater. Chem. B* **2014**, *2*, 1250–1254.
26. Soares, L.A.; Yingzi, W.; Silveira, T.F.S.; Silvestrini, D.R.; Bicalho, U.O.; Filho, N.L.D.; Carmo, D.R. A cubic silsesquioxane modified with purpald®: preparation, characterization and a voltammetric application for determination of sulfite, *Int. J. Electrochem. Sci.* **2013**, *8*, 7565 – 7580.
27. Flodin, J.T.; Lichtenhan, J.D.; Schwab, J.J.; An, Y.-Z.; Fu, X.; Kemp, Z. Biomimetic materials comprising polyhedral oligomeric silsesquioxanes. Patent US 2009/0176006 A1.
28. Sauvet, G.; Fortuniak, W.; Kazmierski, K.; Chojnowski, J. Amphiphilic block and statistical siloxane copolymers with antimicrobial activity. *J. Polym. Sci. A: Polym. Chem.* **2003**, *41*, 2939–2948.
29. Denyer, S.P.; Stewart, G.S.A.B. Mechanisms of action of disinfectants. *Intern. Biodeterior. Biodegrad.* **1998**, *41*, 261–268.
30. Tashiro, T. Antibacterial and Bacterium Adsorbing Macromolecules. *Macromol. Mater. Eng.* **2001**, *286*, 63–87.

31. Kenawy, E.R.; Mahmoud, Y.A.G. Biologically active polymers, 6^a synthesis and antimicrobial activity of some linear copolymers with quaternary ammonium and phosphonium groups. *Macromol. Biosci.* **2003**, *3(2)*, 107–116.
32. Majumdar, P.; Lee, E.; Gubbins, N.; Stafslien, S.J.; Daniels, J.; Thorson, C.J.; Chisholm, B.J. Synthesis and antimicrobial activity of quaternary ammonium-functionalized POSS compounds, *Polymer Preprints* **2008**, *49(1)*, 883.
33. Chojnowski, J.; Fortuniak, W.; Rosciszewski, P.; Werel, W.; Lukasiak, J.; Kamysz, W.; Halasa, R. Polysilsesquioxanes and oligosilsesquioxanes substituted by alkylammonium salts as antibacterial biocides. *J. Inorg. Organomet. Polymer Mater.* **2006**, *16*, 219–230.
34. Majumdar, P.; Lee, E.; Gubbins, N.; Stafslien, S.J.; Daniels, J.; Thorson, C.J.; Chisholm, B.J. Synthesis and antimicrobial activity of quaternary ammonium-functionalized POSS (Q-POSS) and polysiloxane coatings containing Q-POSS. *Polymer* **2009**, *50*, 1124–1133.
35. Majumdar, P.; He, J.; Lee, E.; Kallam, A.; Gubbins, N.; Stafslien, S.J.; Daniels, J.; Chisholm, B.J. Antimicrobial activity of polysiloxane coatings containing quaternary ammonium-functionalized polyhedral oligomeric silsesquioxane. *J. Coat. Technol. Res.* **2010**, *7(4)*, 455–467.
36. Simionescu, B.; Bordianu, I.-E.; Aflori, M.; Doroftei, F.; Mares, M.; Patras, X.; Nicolescu, A.; Olaru, M. Hierarchically structured polymer blends based on silsesquioxane hybrid nanocomposites with quaternary ammonium units for antimicrobial coatings. *Materials Chem. Phys.* **2012**, *134*, 190–199.
37. Liu, Y.; Leng, C.; Chisholm, B.; Stafslien, S.; Majumdar, P.; Chen, Z. Surface structures of PDMS incorporated with quaternary ammonium salts designed for antibiofouling and fouling release applications. *Langmuir* **2013**, *29*, 2897–2905.
38. Ravelin, J. Chemistry of vegetation. *Sic Nat.* **1869**, *11*, 93–102.
39. Berger, T.J.; Spadaro, J.A.; Chapin, S.E.; Becker, R.O. Electrically generated silver ions: quantitative effects on bacterial and mammalian cells. *Antimicrob. Agents Chemother.* **1976**, *9(2)*, 357–358.
40. George, N.; Faoagali, J.; Muller, M. Silvazine (silver sulfadiazine and chlorhexidine) activity against 200 clinical isolates. *Burns* **1997**, *23*, 493–495.
41. Chappel, J.B.; Greville, G.D. Effect of silver ions on mitochondrial adenosine triphosphatase. *Nature* **1954**, *174*, 930–931.
42. Cecil, R.; McPhee, J.R. The sulfur chemistry of proteins. *Adv. Protein Chem.* **1959**, *14*, 255–389.
43. Feng, Q.L.; Wu, J.; Chen, G.Q.; Cui, F.Z.; Kim, T.N.; Kim, J.O. A mechanistic study of the antibacterial effect of silver ions on *Escherichia coli* and *Staphylococcus aureus*. *J. Biomed. Mater. Res*. **2000**, *52,* 662–668.
44. Yang, W.; Shen, C.; Ji, Q.; An, H.; Wang, J.; Liu, Q.; Zhang, Z. Food storage material silver nanoparticles interfere with DNA replication fidelity and bind with DNA. *Nanotechnology* **2009**, *20*, 085102.
45. Raffi, M.; Hussain, F.; Bhatti, T.; Akhter, J.; Hameed, A.; Hasan, M.M. Antibacterial characterization of silver nanoparticles against *Escherichia coli*. *J. Mater. Sci. Technol*. **2008**, *24*, 192–196.

46. Hwang, E.T.; Lee, J.H.; Chae, Y.J.; Kim, Y.S.; Kim, B.C.; Sang, B.-I.; Gu, M.B. Analysis of the toxic mode of action of silver nanoparticles using stress-specific bioluminescent bacteria. *Small* **2008**, *4*, 746–750.
47. Amro, N.A.; Kotra, L.P.; Wadu-Mesthrige, K.; Bulychev, A.; Mobashery, S.; Liu, G.-Y. High resolution atomic force microscopy studies of the *Escherichia coli* outer membrane: structural basis for permeability. *Langmuir* **2000**, *16*, 2789–2796.
48. Sondi, I.; Salopek-Sondi, B. Silver nanoparticles as antimicrobial agent: a case study on *Escherichia coli* as a model for Gram-negative bacteria. *J. Colloid. Interface Sci.* **2004**, *275*, 177–182.
49. Stohs, S.J.; Bagchi, D. Oxidative mechanisms in the toxicity of metal ions. *Free Radic. Biol. Med.* **1995**, *18*, 321–366.
50. Holtz, R.D.; Filho, A.G.S.; Brocchi, M.; Martins, D.; Dura´n, N.; Alves, O.L. Development of nanostructured silver vanadates decorated with silver nanoparticles as a novel antibacterial agent. *Nanotechnology* **2010**, *21*, 185102.
51. Kong, H.; Jang, J. Synthesis and antimicrobial properties of novel silver/polyrhodanine nanofibers. *Biomacromolecules* **2008**, *9*, 2677–2681.
52. Lee, H.Y.; Park, H.K.; Lee, Y.M.; Kim, K.; Park, S.B. A practical procedure for producing silver nanocoated fabric and its antibacterial evaluation for biomedical applications. *Chem. Commun.* **2007**, *28*, 2959–2961.
53. Liu, J.; Sonshine, D.A.; Shervani, S.; Hurt, R.H. Controlled release of biologically active silver from nanosilver surfaces. *ACS Nano* **2010**, *4*, 6903–6913.
54. Zhang, H.; Chen, G. Potent antibacterial activities of Ag/TiO2 nanocomposite powders synthesized by a one-pot sol-gel method. *Environ. Sci. Technol.* **2009**, *43*, 2905–2910.
55. Aflori, M.; Simionescu, B.; Bordianu, I.-E.; Sacarescu, L.; Varganici, C.-D.; Doroftei, F.; Nicolescu, A.; Olaru, M. Silsesquioxane-based hybrid nanocomposites with methacrylate unitscontaining titania and/or silver nanoparticles as antibacterial/antifungal coatings for monumental stones, *Mat. Sci. Eng. B* **2013**, *178*, 1339–1346.
56. Li, Y.; Hindi, K.; Watts, K.M.; Taylor, J.B.; Zhang, K.; Li, Z.; Hunstad, D.A.; Cannon, C.L.; Youngs, W.J.; Wooley, K.L. Shell crosslinked nanoparticles carrying silver antimicrobials as therapeutics. *Chem. Commun.* **2010**, *46*, 121–123.
57. Wu, J.; Hou, S.; Ren, D.; Mather, P.T. Antimicrobial properties of nanostructured hydrogel webs containing silver, *Biomacromolecules* **2009**, *10*, 2686–2693.
58. Chawla, R.; Tan, A.; Ahmed, M.; Crowley, C.; Moiemen, N.S.; Cui, Z.; Butler, P.E.; Seifalian, A.M. A polyhedral oligomeric silsesquioxane- based bilayered dermal scaffold seeded with adipose tissue-derived stem cells: in vitro assessment of biomechanical properties. *J. Surg. Res.* **2014**, *188(2),* 361–372.
59. Zhanga, H.; Oyanedel-Craverb, V. Comparison of the bacterial removal performance of silver nanoparticles and a polymer based quaternary amine functiaonalized silsesquioxane coated point-of-use ceramic water filters. *J. Hazard. Mater.* **2013**, *260*, 272– 277.
60. Schneid, A.C.; Roesch, E.W.; Sperb, F.; Matte, U.; Silveira, N.P.; Costa, T.M.H.; Benvenutti, E.V.; Menezes, E.W. Silver nanoparticle–ionic silsesquioxane: a new system proposed as an antibacterial agent. *J. Mater. Chem. B* **2014**, *2*, 1079–1086.

61. Faundez, G.; Troncoso, M.; Navarrete, P.; Figueroa, G. Antimicrobial activity of copper surfaces against suspensions of *Salmonella enterica* and *Campylobacter*. *BMC Microbial* **2004**, *4*, 19–25.
62. Grass, G.; Rensing, C.; Solioz, M. Metallic copper as an antimicrobial surface. *App. Environ. Microb.* **2011**, *77*, 1541–1547.
63. Pena, M.M.O.; Koch, K.A.; Thiele, D.J. Dynamic regulation of copper uptake and detoxification genes in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **1998**, *18*, 2514–2523.
64. Halliwell, B.; Gutteridge, J.M.C. Oxygen toxicity, oxygen radicals, transition metals and disease. *Biochem. J.* **1984**, *219*, 1–14.
65. Nyden, M.; Fant, C. (2006) PCT Int. Appl. 27 pp. Application: WO 2006-SE318 20060313.
66. Stupp, S.I.; Hartgerink, J.D.; Beniash, E. (2003) PCT Int. Appl. 54 pp. Application: WO 2003-US4779 20030218.
67. Lin, J.; Yang, C.Y.; Chou, C.C.; Su, H.L.; Hung, T.J. (2009) U.S. Pat. Appl. Publ. 27 pp. Application: US 2008–253037 20081016.
68. Taylor, A.; Montoya-Vázquez, M.P.; Avila-Orta, C.A.; Bukowski, M. *Cuvito-the development of novel nanostructured antimicrobial coatings*, 19th International Scientific and Technical Conference, KONTECH, entitled: Advanced forming technologies and nanostructured materials", 06-09 May **2012**, Obróbki Plastycznej Institute, Poznań, Poland.
69. Borrelli, N.F.; Petzold, O.N.; Iii, J.F.S.; Senaratne, W.; Verrier, F.; Wei, Y. Antimicrobial action of Cu, CuO and Cu_2O nanoparticles on glass surfaces and durable coatings, WO2012135294 A2.