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(71) **Applicants**: **NOBIO LTD.** [IL/IL]; 27 Assirei Zion, 4634522 Herzeliya (IL). **HADASIT MEDICAL RESEARCH SERVICES AND DEVELOPMENT LTD.** [IL/IL]; Kiryat Hadassah, P.O. Box 12000, JBP (Minrav) Building Hadassah Ein Kerem, 9112001 Jerusalem (IL).

(72) **Inventors**: **ZALTSMAN, Nathan**; 26/30 Frank St., 3833324 Hadera (IL). **WEISS, Ervin, Itzhak**; 27 Assirei Zion, 4634522 Herzeliya (IL). **BEYTH, Nurit**; Kol Moshe 26, Apartment 11, 9371532 Jerusalem (IL).

(74) **Agents**: **VAN DER BOOM, Tamar** et al.; Pearl Cohen Zedek Latzer Baratz, P.O.Box 12704, Herzliya 4673339 (IL).

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(54) **Title**: MICRO AND NANOPARTICULATE COMPOSITIONS COMPRISING ANTI-MICROBIALLY ACTIVE GROUPS

(57) **Abstract**: The present invention relates to anti-microbially active micro and nanoparticles, compositions comprising same, and use thereof for inhibiting bacterial growth on surfaces or devices, e.g., dental surfaces or devices. The present invention further discloses methods of making such anti-microbially active micro or nanoparticles.

MICRO AND NANOPARTICULATE COMPOSITIONS COMPRISING ANTI-MICROBIALLY ACTIVE GROUPS

FIELD OF THE INVENTION

The present invention relates to anti-microbially active micro and nanoparticles, compositions comprising same, and use thereof for inhibiting bacterial growth on surfaces or devices, e.g., dental surfaces or devices. The present invention further discloses methods of making such anti-microbially active micro or nanoparticles.

BACKGROUND OF THE INVENTION

The overwhelming diversity of bacteria in one individual's skin, gastro intestinal tract and oral cavity is well documented, demonstrating a complex ecosystem anatomically and dynamically in which poly-microbial biofilms are the norm.

Biofilms formed on tissues outside and inside the organism are the major cause of infectious diseases. For example in the oral cavity, dental hard or soft tissue are the major cause of caries and periodontal disease (Sbordone L., Bortolaia C., *Clin Oral Investig* **2003**;7:181–8). Bacterial biofilm forms on both natural and artificial dental surfaces.

Special attention is paid in recent years to artificial surfaces contacting organisms, as these surfaces lack the epithelial shedding, a major natural mechanism to combat biofilms, thus biofilm accumulation is becoming a major source of medical complications that may result in life threatening problems. Two major factors influence the susceptibility of a surface to accumulate bacteria: surface roughness and the surface-free energy which is a property of the material used. Surface roughness has a higher influence on the adhesion of bacteria than surface-free energy. In this context, artificial restorative materials typically have a higher surface roughness than natural dental surfaces, and therefore are more prone to bacterial accumulation. For example, in the oral cavity, biofilm formation on dental filling material such as composite resins causes material degradation and can also result in bacterial invasion to the tooth-resin interface, leading to secondary caries or even pulp infection.

Persisting biofilms in the endodontically treated root canal may lead to periapical or periodontal diseases. Biofilms on dental implants may cause peri-implantitis. Therefore, the development of new materials that diminishes biofilm formation is a critical topic chronic infectious disease control, in various sites of the human body.

The ultimate goal of the development of dental materials with antibiofilm properties is to improve health and reduce disease occurrence. None of the existing medical devices can guarantee immediate and comprehensive elimination of biofilm or prevention of secondary infection. In order to sustain the oral defense, dental materials with the following antibiofilm properties are sought after: (1) inhibition of initial binding of microorganisms (2) preventing biofilm growth, (3) affecting microbial metabolism in the biofilm, (4) killing biofilm bacteria, and (5) detaching biofilm (Busscher HJ, Rinastiti M, Siswomihardjo W, van der Mei HC., *J Dent Res*, **2010**;89:657–65; Marsh PD. *J Dent*, **2010**;38).

Resin-based composites are complex materials that consist of a hydrophobic resin matrix and less hydrophobic filler particles, which implies that a resin-based composite surface is never a homogeneous interface but rather one that produces matrix-rich and filler-poor areas, as well as matrix-poor and filler-rich areas (Ionescu A, Wutscher E, Brambilla E, Schneider-Feyrer S, Giessibl FJ, Hahnel S.; *Eur J Oral Sci* **2012**;120:458–65).

Biofilms on composites can cause surface deterioration. Polishing, as well as differences in the composition of the resin-based composite, may have an impact on biofilm formation on the resin-based composite surface (Ono M. et al., *Dent Mater J*, **2007**;26:613–22).

Surface degradation of resin composites driven by polishing leads to increased roughness, changes in micro hardness, and filler particle exposure upon exposure to biofilms in vitro.

U.S. Patent No. 8,535,645 to some of the inventors of the present invention discloses aliphatic polymer-based particles directly bonded to a quaternary ammonium group, the nitrogen of each quaternary comprising one alkyl group having from 4 to 10 carbon atoms, demonstrating anti-microbial activity.

There still remains a need for and it would be advantageous to have an extended variety of anti-microbially active materials which are cost-effective, non-

toxic and easy to apply to contaminated surfaces and devices, especially in dental products.

SUMMARY OF THE INVENTION

The present invention provides anti-microbially active functionalized particles, which can be embedded in a matrix to form compositions demonstrating a broad spectrum of anti-microbial activity. The compositions of the invention are preferably formulated for topical administration and can prevent the formation of biofilm on surfaces and devices, e.g., dental surfaces and devices. Furthermore, the present invention provides versatile and cost-effective methodology for the preparation of the anti-microbially active particles of the invention.

The present invention is based on the surprising discovery that microparticles or nanoparticles comprising an inorganic or organic core, and anti-microbially active groups chemically bound to the core at a surface density of at least one anti-microbially active group per 10 sq. nm, show a broad spectrum of anti-microbial activity when applied to surfaces and devices on which the growth of such microbes may otherwise naturally take place. Such anti-microbial activity thus prevents biofilm formation. The particles generally include a core which can be made of an organic polymeric material or inorganic materials, as described herein. Depending on the nature of the core, the anti-microbially active group may be selected from: (a) a tertiary amine comprising at least one terpenoid moiety and optionally an alkyl group having from 1 to 4 carbon atoms, or a salt of said amine; (b) a quaternary ammonium group comprising at least one terpenoid moiety and optionally one or more alkyl groups having from 1 to 4 carbon atoms; and (c) a quaternary ammonium group, the nitrogen atom of each quaternary ammonium group having one bond to an alkyl group having from 4 to 18 carbon atoms, and a remainder of bonds each being to an alkyl group having from 1 to 3 carbon atoms. It was further surprisingly discovered that these microparticles and nanoparticles maintain high anti-microbial properties over time without leaching out and with no alteration of the properties of the hosting matrix.

The particles of the present invention demonstrate enhanced anti-bacterial activity originating from the presence of closely packed anti-bacterial groups on a

given particle's surface. This effect yields a high local concentration of active functional groups (at least one anti-microbially active group per 10 sq. nm, preferably at least one anti-microbially active group per 1 sq. nm), which results in high effective concentration of the functionalized particles and enables the use of a relatively small amount of particles to achieve effective bacterial annihilation.

Thus, according to one aspect, the present invention provides a particle comprising: (i) an inorganic core or an organic polymeric core; and (ii) anti-microbially active groups chemically bound to the core at a surface density of at least one anti-microbially active group per 10 sq. nm, wherein the anti-microbially active group is: (a) a tertiary amine comprising at least one terpenoid moiety and optionally an alkyl group having from 1 to 4 carbon atoms, or a salt of said amine; or (b) a quaternary ammonium group comprising at least one terpenoid moiety and optionally one or more alkyl groups having from 1 to 4 carbon atoms.

In another aspect, the present invention provides a particle comprising: (i) an organic polymer core; and (ii) anti-microbially active groups chemically bound to the core at a surface density of at least one anti-microbially active group per 10 sq. nm, wherein the anti-microbially active group is selected from the group consisting of: (a) a tertiary amine comprising at least one terpenoid moiety and optionally an alkyl group having from 1 to 4 carbon atoms, or a salt of said amine; and (b) a quaternary ammonium group comprising at least one terpenoid moiety and optionally one or more alkyl groups having from 1 to 4 carbon atoms.

In another aspect, the present invention provides a particle comprising: (i) an inorganic core; and (ii) anti-microbially active groups chemically bound to the core at a surface density of at least one anti-microbially active group per 10 sq. nm, wherein the anti-microbially active group is selected from the group consisting of: (a) a tertiary amine comprising at least one terpenoid moiety and optionally an alkyl group having from 1 to 4 carbon atoms, or a salt of said amine, wherein the terpenoid moiety is a bornyl group derived from camphor, bornyl halide or bornyl alcohol, or wherein the terpenoid moiety is derived from citral or perilaldehyde; and (b) a quaternary ammonium group comprising at least one terpenoid moiety and optionally one or more alkyl groups having from 1 to 4 carbon atoms

In yet another aspect, the present invention provides a particle comprising (i) an inorganic core selected from silicate (SiO_4^{-4}), surface activated metal and metal oxide; and (ii) anti-microbially active groups chemically bound to the core at a surface density of at least one anti-microbially active group per 10 sq. nm, wherein the anti-microbially active group is selected from the group consisting of: (a) a tertiary amine comprising at least one terpenoid moiety and optionally an alkyl group having from 1 to 4 carbon atoms, or a salt of said amine; and (b) a quaternary ammonium group comprising at least one terpenoid moiety and optionally one or more alkyl groups having from 1 to 4 carbon atoms.

In another aspect, the present invention provides a particle comprising: (i) an inorganic core or an organic polymeric core; and (ii) anti-microbially active groups chemically bound to the core at a surface density of at least one anti-microbially active group per 10 sq. nm, wherein the anti-microbially active group is a quaternary ammonium group including at least one terpenoid moiety and optionally one or more alkyl groups having from 1 to 4 carbon atoms.

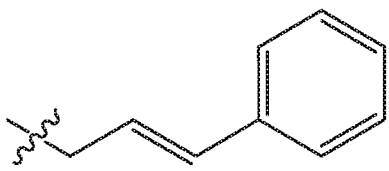
In another embodiment, the anti-microbially active group contains at least one terpenoid group, and is selected from: (a) a tertiary amine, the nitrogen atom of each tertiary amine having at least one bond to the core, one bond to a terpenoid moiety and optionally remaining bond to an alkyl group having from 1 to 4 carbon atoms or a salt of said tertiary amine; (b) a tertiary amine, the nitrogen atom of each tertiary amine having one bond to the core, and two bonds to terpenoid moieties which may be the same or different from each other, or a salt of said tertiary amine; and (c) a quaternary ammonium group, the nitrogen atom of each quaternary ammonium group having at least one bond to the core, one or two bonds to terpenoid moieties which may be the same or different from each other, and optionally remaining bonds to an alkyl group having from 1 to 4 carbon atoms. Each possibility represents a separate embodiment of the present invention.

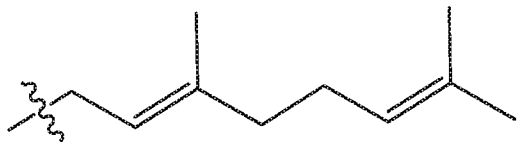
In additional embodiments, the particle is in the form of a protonated ammonium salt, having at least one counter-ion.

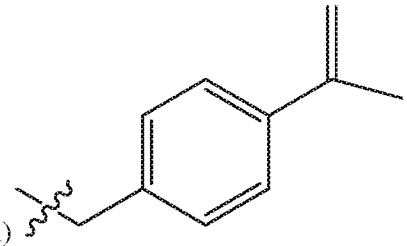
In one embodiment, the at least one terpenoid moiety is a cinammyl group derived from cinnamaldehyde, cinnamic acid or cinnamyl alcohol. In another embodiment, the at least one terpenoid moiety is a bornyl group derived from

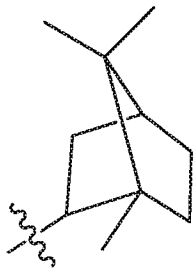
camphor, bornyl halide or bornyl alcohol. In another embodiment, the at least one terpenoid moiety is derived from citral. In another embodiment, the at least one terpenoid moiety is derived from perilaldehyde. Each possibility represents a separate embodiment of the present invention.

In some embodiments, the at least one anti-microbially active terpenoid moiety comprises a functional group selected from the group consisting of:

(i)  ;

(ii)  ;

(iii)  ; and

(iv)  .

Each possibility represents a separate embodiment of the present invention.

In yet another aspect, the present invention provides a particle comprising: (i) an inorganic core; and (ii) anti-microbially active groups chemically bound to the core at a surface density of at least one anti-microbially active group per 10 sq. nm, wherein the anti-microbially active group is a quaternary ammonium group, the nitrogen atom of each quaternary ammonium group having one bond to an alkyl group

having from 4 to 18 carbon atoms, and a remainder of bonds each being to an alkyl group having from 1 to 3 carbon atoms.

In another aspect, the present invention provides a particle comprising: (i) an inorganic core selected from silicate (SiO_4^{-4}), surface activated metal and metal oxide; and (ii) anti-microbially active groups chemically bound to the core at a surface density of at least one anti-microbially active group per 10 sq. nm, wherein the anti-microbially active group is a quaternary ammonium group, the nitrogen atom of each quaternary ammonium group having one bond to an alkyl group having from 4 to 18 carbon atoms, and a remainder of bonds each being to an alkyl group having from 1 to 3 carbon atoms.

It is understood that the quaternary ammonium group is positively charged, and is counter-balanced by an anion, for example a fluoride (F^-).

In one embodiment, the anti-microbially active group is chemically bound to the core at a surface density of at least one anti-microbially active group per 1 sq. nm.

In other embodiments, the nitrogen atom of each quaternary ammonium group has (i) at least one bond to the core; (ii) one bond to the alkyl group having from 4 to 18 carbon atoms, and (iii) the remainder of the bonds each being to an alkyl group having from 1 to 3 carbon atoms.

In some currently preferred embodiments, the alkyl group having from 4 to 18 carbon atoms is an alkyl group having 4 to 10 carbon atoms. In other currently preferred embodiments, the alkyl group having from 4 to 18 carbon atoms is an alkyl group having 6, 7, or 8 carbon atoms, with each possibility representing a separate embodiment of the present invention. In other currently preferred embodiments, the alkyl group having from 1 to 3 carbon atoms is a methyl group.

Depending on the nature of the anti-microbially active group, the core of the particles may be an organic polymeric core (i.e., in the case of tertiary amines or quaternary ammonium groups comprising at least one terpenoid moiety), or an inorganic core (i.e., in the case of tertiary amines, or quaternary ammonium groups comprising at least one terpenoid moiety or quaternary ammonium groups comprising one alkyl group having from 4 to 18 carbon atoms).

In some embodiments, the core of the particles is an organic polymeric core. Non-limiting examples of such polymeric core include: (a) an organic core having at

least one aliphatic polymer selected from the following group: polyethylene imine (PEI), polyvinyl amine (PVA), poly(allyl amine) (PAA), poly(aminoethyl acrylate), polypeptides with pending alkyl-amino groups, and chitosan, preferably, wherein the polymer is polyethylene imine (PEI); or (b) at least one aromatic polymer selected from the following group: aminomethylated styrene polymers, aromatic polyesters, preferably polyethylene terephthalate, and polyvinyl pyridine. The polymeric core may be linked to the anti-microbially active group directly or through a linker. Each possibility represents a separate embodiment of the present invention.

In some embodiments, the anti-microbially active group is linked to the polymeric core through a linker derived from: (a) amino acid of natural or synthetic source having a chain length of between 2 and 18 carbons wherein the carboxyl end is attached to the polymer core and the amino end is attached to the antibacterial functional group; (b) halo- acyl halides of said acids with a chain length of between 2 and 18 carbons, wherein the carboxyl end is attached to the polymer core and the amino end to the antibacterial functional group; (c) di-halo-alkyl having a carbon chain of between 2 and 18 carbons substituted by two halides situated on different carbon atoms, preferably on opposite ends of the alkyl group; and (d) aromatic molecules selected from 4,4-biphenol, dibenzoic acid, dibenzoic halides, dibenzoic sulphonates, terephthalic acid, tetrphthalic halides, and terephthalic sulphonates. Each possibility represents a separate embodiment of the present invention.

In one specific embodiment, the polymeric core is cross-linked with a cross-linking agent. The degree of cross-linking may be from about 1% to about 20%.

In some embodiments, at least about 10% of the amine groups in the organic polymeric core are the anti-microbially active tertiary amine or quaternary ammonium groups or salts thereof.

In some embodiments, the core of the particles is an inorganic core comprising one or more of the following: (a) silica (SiO_2) in a form selected from the following group: amorphous silica, dense silica, aerogel silica, porous silica, mesoporous silica and fumed silica; (b) glasses or ceramics of silicate (SiO_4^{-4}) selected from the following group: aluminosilicate, borosilicate, barium silicate, barium borosilicate and strontium borosilicate; (c) surface activated metals selected from the group of: silver, gold, platinum, palladium, copper, zinc and iron; or (d) metal oxides selected from the

group of: zirconium dioxide, titanium dioxide, vanadium dioxide, zinc oxide, copper oxide and magnetite.

The inorganic core typically has a solid uniform morphology with low porosity or a porous morphology having pore size diameter of between about 1 to about 30 nm.

Depending on the chemical nature of the inorganic core, the core may be attached to the anti-microbially active group directly or through a linker. Preferably a silica (SiO_2) based inorganic core may be attached to the anti-microbially active group through a linker, while silicates (SiO_4^{-4}), metals or metal oxides may be attached to the anti-microbially active group directly or through a linker.

In some embodiments, the inorganic core is directly attached to the anti-microbially active group. In other embodiments, the inorganic core is attached to the anti-microbially active group through a linker. In some embodiments, the linker is selected from the following groups: a C3 to C18 alkylene substituted with at least one silane moiety; a C3 to C18 alkylene substituted with at least one phosphate moiety; a C3 to C18 alkylene substituted with at least one anhydride moiety; a C3 to C18 alkylene substituted with at least one carboxylate moiety; and a C3 to C18 alkylene substituted with at least one glycidyl moiety. Each possibility represents a separate embodiment of the present invention.

As contemplated herein, the inorganic core – linker – anti-microbially active group adduct may be formed using a variety of reagents. The choice of the reagent depends on the nature of the anti-microbially active group. For example, when the anti-microbially active group is a tertiary amine or a quaternary ammonium group comprising at least one terpenoid moiety, a preferred reagent for coupling the inorganic core to the anti-microbially active group is represented by the structure of formula (I). Alternatively, when the anti-microbially active group is a quaternary ammonium group containing one alkyl group having 4 to 18 carbon atoms, a preferred reagent for coupling the inorganic core to the anti-microbially active group is represented by the structure of formula (II). The structures of formula (I) and formula (II) are presented in the detailed description.

The inorganic core of the particle as described above may generally be in a form selected from a sphere, amorphous polygonal, shallow flake-like and a rod. In some representative embodiments, the inorganic core is spherical and has a diameter

between about 5 to about 100,000 nm with pore diameter of about 1 to about 30 nm. In another embodiment, the inorganic particle is in a form of a rod, having a diameter of between about 10 to about 1,000 nm and length between about 10 to about 1,000,000 nm and a pore diameter of about 1 to about 30 nm. Each possibility represents a separate embodiment of the present invention.

The particles of the present invention are characterized by having a diameter between about 5 to about 100,000 nm. Preferred are particles between about 10 to about 50,000 nm.

According to another aspect, the present invention provides a composition having a liquid or solid matrix embedding a plurality of particles as described above, wherein the particles are embedded in the matrix through covalent or non-covalent interactions.

In one embodiment, the composition includes a polymeric matrix having a thermoplastic polymer selected from the following group: polyethylene, polypropylene, silicone, epoxy, composite materials and acrylic polymers such as poly methyl methacrylate. Each possibility represents a separate embodiment of the present invention.

In another embodiment, the composition is characterized by having homogeneously distributed particles on the outer surface of the matrix at a surface concentration of between about 1 to about 100 particles per sq. μm. The high density of the anti-microbially active groups on the surface of the core, together with the high surface concentration of the particles on the surface of the matrix results in high effective concentration of the functionalized particles and enables the use of a relatively small amount of particles to achieve effective bacterial annihilation.

In yet another embodiment, the composition is characterized by having, on the average, at least one active portion per sq. μm of outer surface of matrix, the size of such active portion is at least 100 nm^2.

In some embodiments, the composition is a pharmaceutical composition. In some preferred embodiments, the composition is formulated as a topical composition. For example, the composition may be in a form selected from: a cream, an ointment, a paste (e.g., a toothpaste), a dressing and a gel. Each possibility represents a separate embodiment of the present invention.

Particles comprising an organic core and as an anti-microbially active group a quaternary ammonium group containing one alkyl group having 4 to 10 carbon atoms, were described in US 8,535,645. However, US 8,535,645 does not describe topical formulations such as creams, ointments, etc., or particles having a diameter between about >10,000 nm and about 100,000 nm. Such particles are new and represent another embodiments of the present invention.

Thus, in some embodiments, the present invention relates to a pharmaceutical composition formulated for topical administration comprising (i) an organic core; and (ii) anti-microbially active groups chemically bound to the core at a surface density of at least one anti-microbially active group per 10 sq. nm, wherein the anti-microbially active group is a quaternary ammonium group, the nitrogen atom of each quaternary ammonium group having one bond to an alkyl group having from 4 to 18 carbon atoms, and a remainder of bonds each being to an alkyl group having from 1 to 3 carbon atoms; wherein the composition is in a form selected from the group consisting of a cream, an ointment or a paste. U.S. 8,535,645 does not describe pharmaceutical compositions for topical use.

In other embodiments, the present invention provides a particle comprising (i) an organic core; and (ii) anti-microbially active groups chemically bound to the core at a surface density of at least one anti-microbially active group per 10 sq. nm, wherein the anti-microbially active group is a quaternary ammonium group, the nitrogen atom of each quaternary ammonium group having one bond to an alkyl group having from 4 to 18 carbon atoms, and a remainder of bonds each being to an alkyl group having from 1 to 3 carbon atoms; wherein the diameter of each particle is between about >10,000 nm and about 100,000 nm. U.S. 8,535,645 does not describe particle sizes of this range.

According to other aspects, the present invention provides a method for inhibiting or preventing biofilm formation, including the step of applying onto a susceptible or infected surface or a medical device a particle according to the present invention, or a pharmaceutical composition as described above.

In one embodiment, the composition is intended for administration into the oral cavity. In other embodiments, the composition may be formulated as a tooth paste, and/or applied to a surface or medical device selected from the group consisting of: a denture cleaner, post hygienic treatment dressing or gel, mucosal adhesive paste,

a dental adhesive, a dental restorative composite based material for filling tooth, decay cavities, a dental restorative endodontic filling material for filling root canal space in root canal treatment, a dental restorative material used for provisional and final tooth restorations or tooth replacement, a dental inlay, a dental onlay, a crown, a partial denture, a complete denture, a dental implant and a dental implant abutment. Each possibility represents a separate embodiment of the present invention.

In another embodiment, the present invention provides a particle or a pharmaceutical composition comprising such particle as described above for use in inhibiting or preventing a biofilm formation.

In yet another embodiment, the present invention provides a method for inhibiting bacteria by contacting the bacteria with a particle or a composition comprising such particle as described above. In some embodiments, the anti-bacterial compositions of the present invention affect annihilation of at least about 95% of the contacted bacteria, preferably, at least about 99% of the contacted bacteria.

Further embodiments and the full scope of applicability of the present invention will become apparent from the detailed description given hereinafter. However, it should be understood that the detailed description and examples, while indicating preferred embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE FIGURES

FIGURE 1: depicts the anti-microbial activity of a polypropylene matrix without (PP) and with 1% wt/wt (PP + 1% NPs) or 2% wt/wt (PP + 2% NPs) silica particles functionalized with dimethyl octyl ammonium groups, against the Graham positive bacteria Staphylococcus aureus (S. aureus). The embedded particles were 186 nm in diameter on average, and the results were compared with the natural growth of S. aureus.

FIGURE 2: depicts the anti-microbial activity of a polypropylene matrix without (PP) and with 1% wt/wt (PP + 1% NPs) and 2% wt/wt (PP + 2% NPs) silica particles functionalized with dimethyl octyl ammonium groups, against the Graham negative

bacteria Pseudomonas aeruginosa (P. aeruginosa). The embedded particles were 186 nm in diameter on average, and the results were compared with the natural growth of P. aeruginosa.

FIGURE 3: depicts the anti-microbial activity of a poly(methyl methacrylate) matrix without (PMMA) and with 1% wt/wt silica core particles functionalized with quaternary dimethyl octyl ammonium groups (PMMA + 1% Particles), against the Graham negative bacteria Pseudomonas aeruginosa (P. aeruginosa). The embedded particles were 13 µm in diameter on average, and the results were compared with the natural growth of P. aeruginosa.

FIGURE 4: depicts the anti-microbial activity of a poly(methyl methacrylate) matrix without (PMMA) and with 1% wt/wt silica core particles functionalized with quaternary dimethyl octyl ammonium groups (PMMA + 1% Particles), against the Graham positive bacteria Staphylococcus aureus (S. aureus). The embedded particles were 13 µm in diameter on average, and the results were compared with the natural growth of S. aureus.

FIGURE 5: depicts the anti-microbial activity of a poly(methyl methacrylate) matrix without (PMMA) and with silica core particles functionalized with di-cinnamyl amine groups (PMMA + 1% NPs), against the Graham negative bacteria Pseudomonas aeruginosa (P. aeruginosa). The embedded particles were 186 nm in diameter on average, and the results were compared with the natural growth of P. aeruginosa.

FIGURE 6: depicts the anti-microbial activity of a poly(methyl methacrylate) matrix without (PMMA) and with silica core particles functionalized with di-cinnamyl amine groups (PMMA + 1% NPs), against the Graham positive bacteria Staphylococcus aureus (S. aureus). The embedded particles were 186 nm in diameter on average, and the results were compared with the natural growth of S. aureus.

FIGURE 7: depicts the anti-microbial activity of a poly(methyl methacrylate) matrix without (PMMA) and with 1% wt/wt (PMMA + 1% NPs) or 2% wt/wt (PMMA + 2% NPs) Magnetite (Fe_3O_4) core particles functionalized with quaternary dimethyl octyl ammonium groups, against the Graham positive bacteria Enterococcus faecalis (E. faecalis). The embedded particles were 78 nm in diameter on average, and the results were compared with the natural growth of E. faecalis.

FIGURE 8: depicts the anti-microbial activity of a poly(methyl methacrylate) matrix without (PMMA) surface and with 2% wt/wt (PMMA + 2% NPs) or 3% wt/wt (PMMA + 3% NPs) silica core particles functionalized with di-cinnamyl methyl ammonium groups against the Graham positive bacteria Enterococcus faecalis (E. faecalis). The embedded particles were 186 nm in diameter on average, and the results were compared with the natural growth of E. faecalis.

FIGURE 9: mechanical properties test measuring the young's modulus of modified polymer including functionalized antibacterial particles in comparison to unmodified polymer. A) an image of the cylindrical specimens; B) compressive strength test of modified and unmodified specimens.

FIGURE 10: depicts the anti-microbial activity of modified and unmodified specimens of Unifast Trad (a self-cured, methylmethacrylate resin), prepared without (Unifast) or with 8% nanoparticles (NPs): silica + quaternary dimethyl octyl ammonium group (QSi) and PEI + quaternary dimethyl octyl ammonium (QPEI). A) anti-microbial activity against the Graham positive bacteria E. faecalis. The results were compared with the natural growth of E. faecalis. B) anti-microbial activity against the Graham positive bacteria S. aureus. The results were compared with the natural growth of S. aureus.

FIGURE 11: demonstrates anti-microbial activity as evaluated by an imprint method on blood agar. The samples measured are: 1) dimethylamine functionalized silica particles; and 2) tertiary amine with two cinnamyl groups functionalized silica particles.

DETAILED DESCRIPTION OF THE INVENTION

Particles

The present invention provides anti-microbially active functionalized micro or nanoparticles, which can be embedded in a matrix to form compositions demonstrating a broad spectrum of anti-microbial activity. The particles generally include a core which can be made of an organic polymeric material or inorganic materials, as described herein. Depending on the nature of the core, the anti-microbially active group may be selected from: (a) a tertiary amine comprising at least one terpenoid moiety and optionally an alkyl group having from 1 to 4 carbon atoms,

or a salt of said amine; (b) a quaternary ammonium group comprising at least one terpenoid moiety and optionally one or more alkyl groups having from 1 to 4 carbon atoms; and (c) a quaternary ammonium group, the nitrogen atom of each quaternary ammonium group having one bond to an alkyl group having from 4 to 18 carbon atoms, and a remainder of bonds each being to an alkyl group having from 1 to 3 carbon atoms.

Depending on the nature of the anti-microbially active group, the core of the particles may be an organic polymeric core (i.e., in the case of tertiary amines or quaternary ammonium groups comprising at least one terpenoid moiety), or an inorganic core (i.e., in the case of tertiary amines, or quaternary ammonium groups comprising at least one terpenoid moiety or quaternary ammonium groups comprising one alkyl group having from 4 to 18 carbon atoms).

The particles of the present invention demonstrate an enhanced anti-bacterial activity originating from the presence of closely packed anti-bacterial groups on a given particle's surface, as well as high density of particles packed on the surface of a host matrix. The surface density of the anti-microbial group results in high effective concentration promoting anti-bacterial inhibitory effect. According to the principles of the present invention, high surface density dictates high anti-microbial efficiency.

The anti-microbially active groups of the present invention are chemically bound to the core at a surface density of at least one anti-microbially active group per 10 sq. nm. In one preferred embodiment, the surface of the particle includes at least 1 anti-microbially active quaternary ammonium group per sq. density.

The term "nanoparticle" as used herein refers to a particle having a diameter of less than about 1,000 nm. The term "microparticle" as used herein refers to a particle having a diameter of about 1,000 nm or larger.

The particles of the present invention are characterized by having a diameter between about 5 to about 100,000 nm, and thus encompass both nanoparticulate and microparticulate compositions. Preferred are particles between about 10 to about 50,000 nm. In other embodiments, the particles are more than 1,000 nm in diameter. In other embodiments, the particles are more than 10,000 nm in diameter. In other embodiment, the particles are between 1,000 and 50,000 nm in diameter. It is apparent

to a person of skill in the art that other particles size ranges are applicable and are encompassed within the scope of the present invention.

Anti-microbially active amines comprising terpenoid groups

In one embodiment, the anti-microbially active group of the present invention contains at least one terpenoid group, and is selected from: (a) a tertiary amine comprising at least one terpenoid moiety and optionally an alkyl group having from 1 to 4 carbon atoms, or a salt of said amine; (b) a quaternary ammonium group comprising at least one terpenoid moiety and optionally one or more alkyl groups having from 1 to 4 carbon atoms;

In some embodiments, the anti-microbially active group is selected from: (a) a tertiary amine, the nitrogen atom of each tertiary amine having at least one bond to the core, one bond to a terpenoid moiety and optionally remaining bond to an alkyl group having from 1 to 4 carbon atoms or a salt of said tertiary amine; (b) a tertiary amine, the nitrogen atom of each tertiary amine having one bond to the core, and two bonds to terpenoid moieties which may be the same or different from each other, or a salt of said tertiary amine; and (c) a quaternary ammonium group, the nitrogen atom of each quaternary ammonium group having at least one bond to the core, one or two bonds to terpenoid moieties which may be the same or different from each other, and optionally remaining bonds to an alkyl group having from 1 to 4 carbon atoms. Each possibility represents a separate embodiment of the present invention.

The term "terpenoid", also known as "isoprenoid" refers to a large class of naturally occurring compounds that are derived from five-carbon isoprene units.

In one embodiment, the at least one terpenoid moiety is a cinammyl group derived from cinnamaldehyde, cinnamic acid or cinnamyl alcohol. In another embodiment, the at least one terpenoid moiety is a bornyl group derived from camphor, bornyl halide or bornyl alcohol. In another embodiment, the at least one terpenoid moiety is derived from citral. In another embodiment, the at least one terpenoid moiety is derived from perilaldehyde. Each possibility represents a separate embodiment of the present invention.

Cinnamaldehyde is a natural aldehyde extracted from the genus *Cinnamomum*. It is known for its low toxicity and its effectiveness against various bacteria and fungi.

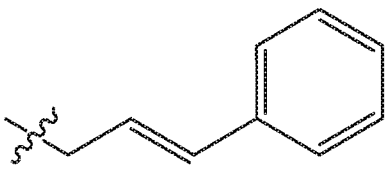
Camphor is found in the wood of the camphor laurel (*Cinnamomum camphora*), and also of the kapur tree. It also occurs in some other related trees in the laurel family, for example *Ocotea usambarensis*, as well as other natural sources. Camphor can also be synthetically produced from oil of turpentine.

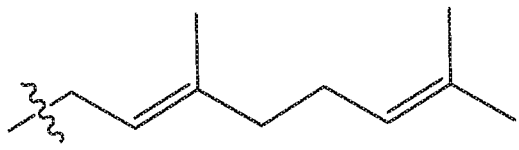
Citral, or 3,7-dimethyl-2,6-octadienal or lemonal, is a mixture of two diastereomeric terpenoids. The two compounds are double bond isomers. The E-isomer is known as geranial or citral A. The Z-isomer is known as neral or citral B. Citral is known to have anti-bacterial activity.

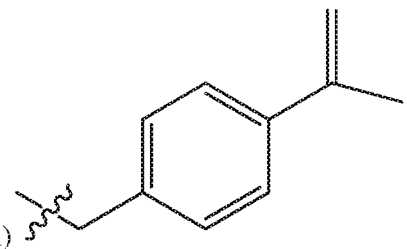
Perillaldehyde, also known as perilla aldehyde, is a natural terpenoid found most in the annual herb perilla, as well as in a wide variety of other plants and essential oils.

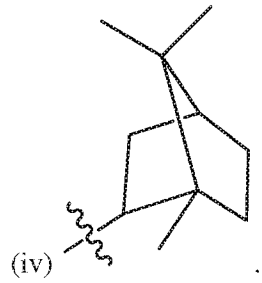
Other examples of terpenoids include, but are not limited to: curcuminoids found in turmeric and mustard seed, and citronellal found in Cymbopogon (lemon grass). Each possibility represents a separate embodiment of the present invention.

In accordance with the above embodiment, the one anti-microbially active terpenoid moiety comprises a functional group selected from the group consisting of:

(i)  ;

(ii)  ;

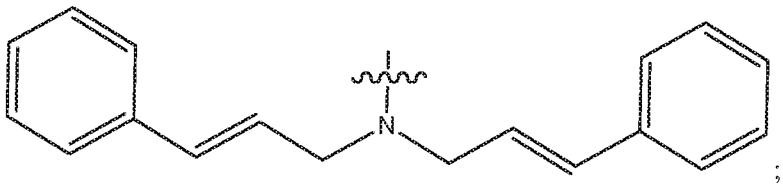
(iii)  ; and



(iv) .

Each possibility represents a separate embodiment of the present invention.

Non-limiting examples of functional anti-microbially active tertiary amine groups in accordance with the principles of the present invention are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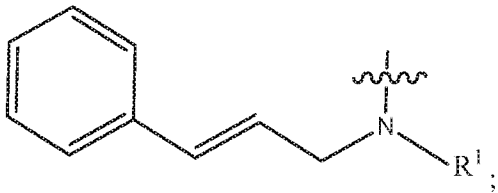
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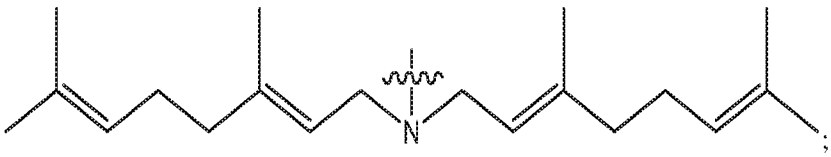
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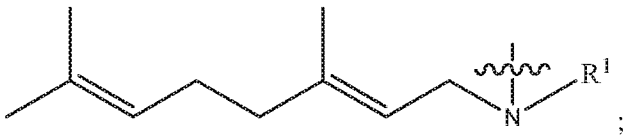
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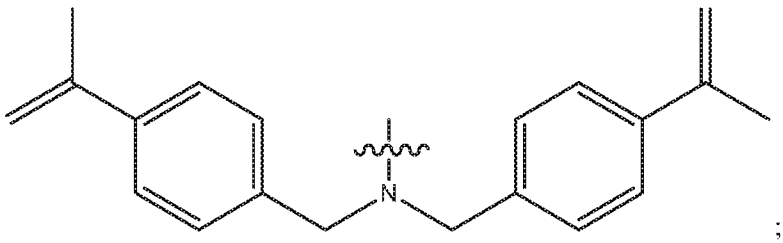
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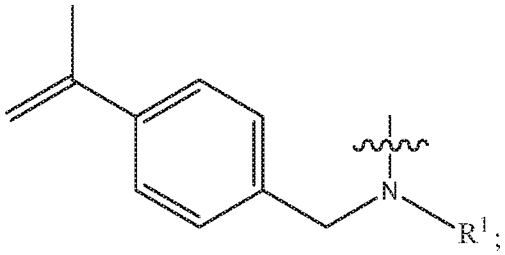

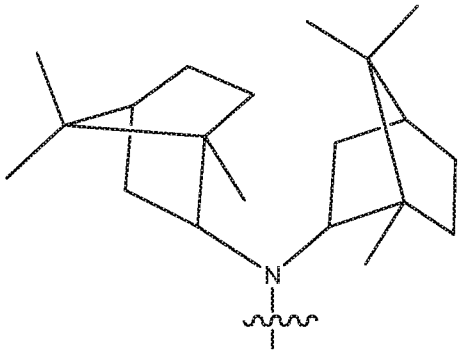

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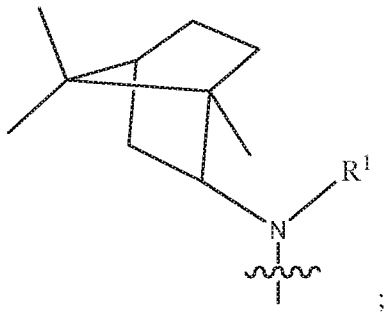

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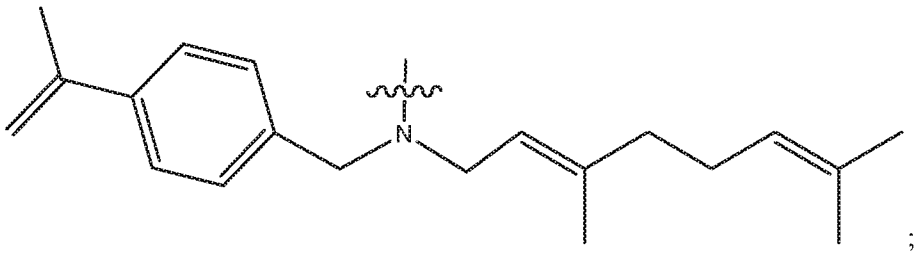

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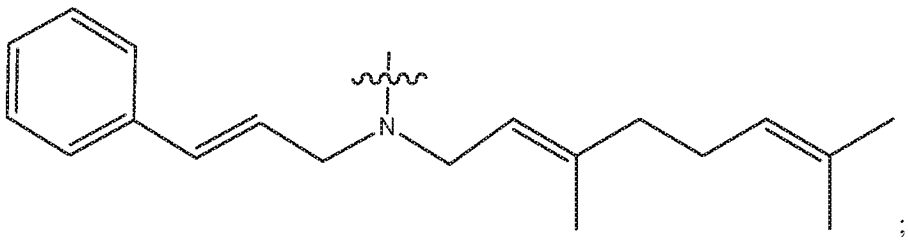

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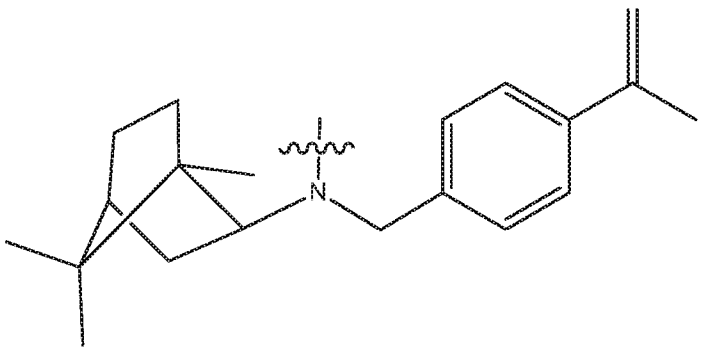

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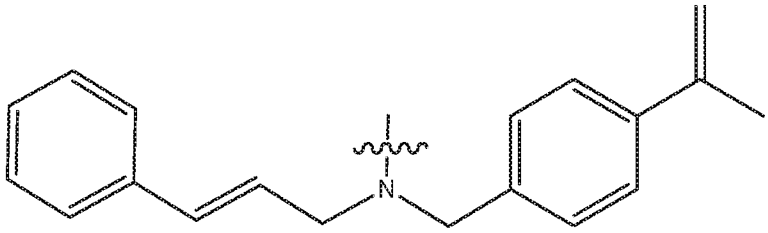

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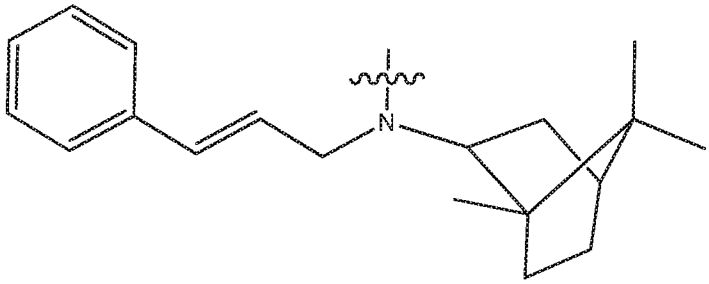

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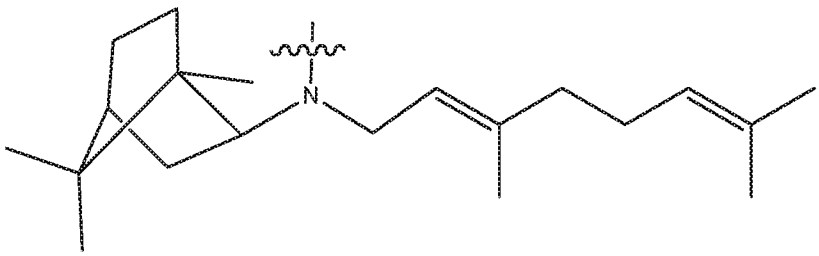

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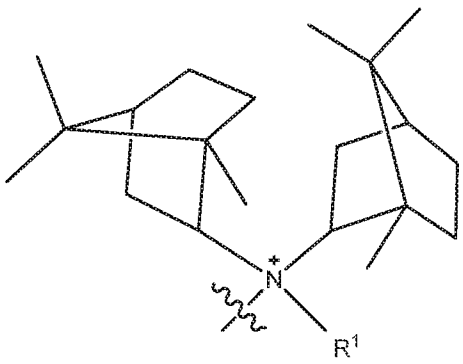
wherein R^1 is an alkyl group having from 1 to 4 carbon atoms.

Non-limiting examples of functional anti-microbially active quaternary ammonium groups in accordance with the principles of the present invention are



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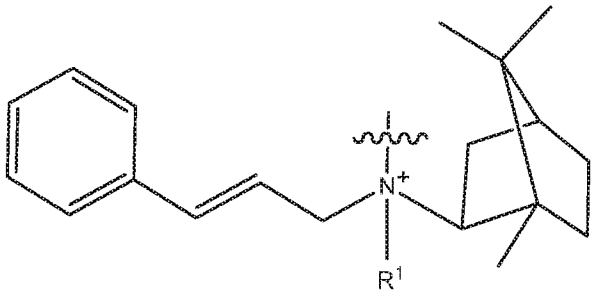

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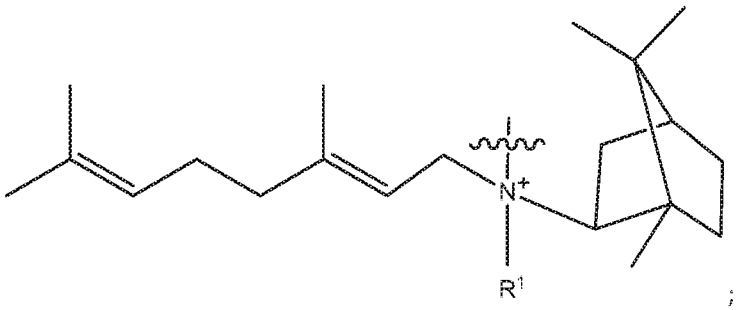

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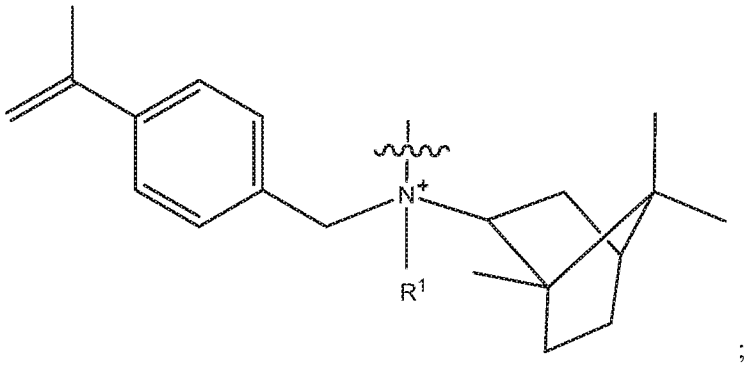

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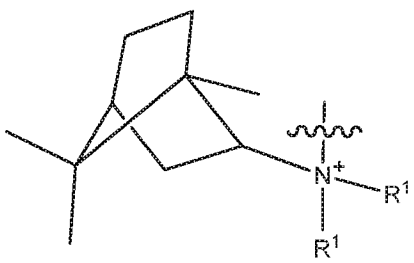

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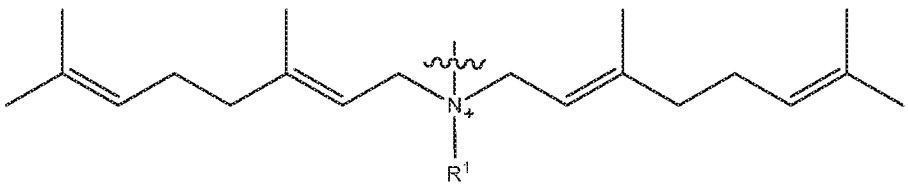

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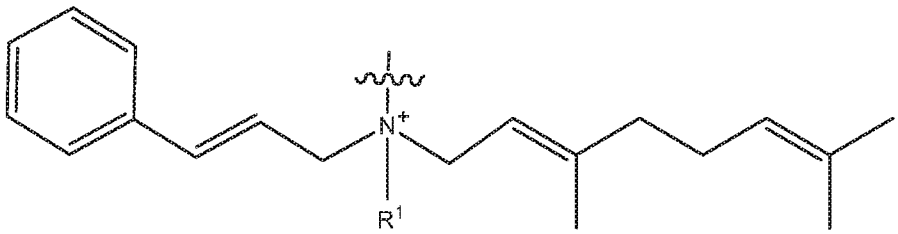
N+
R1

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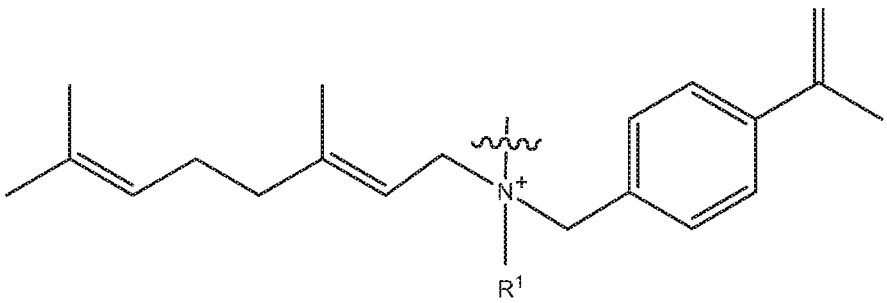
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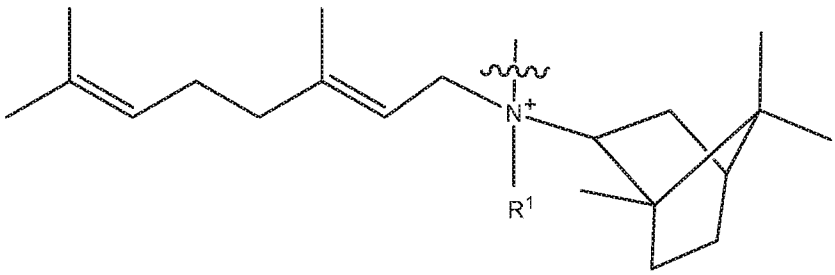
N+
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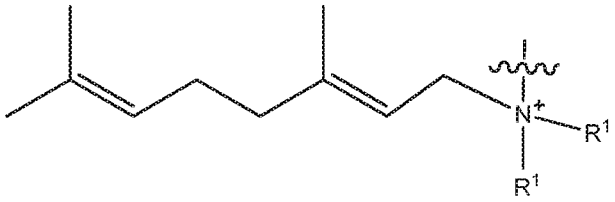
N+
R1
R1

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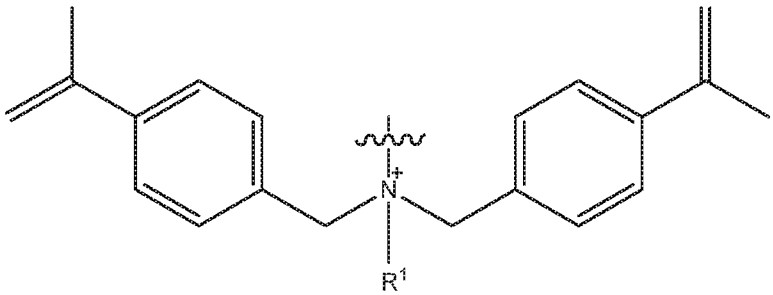
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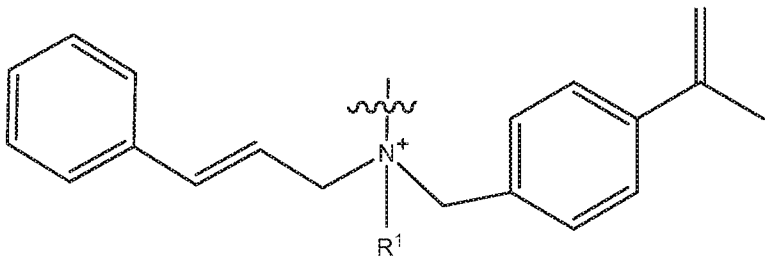
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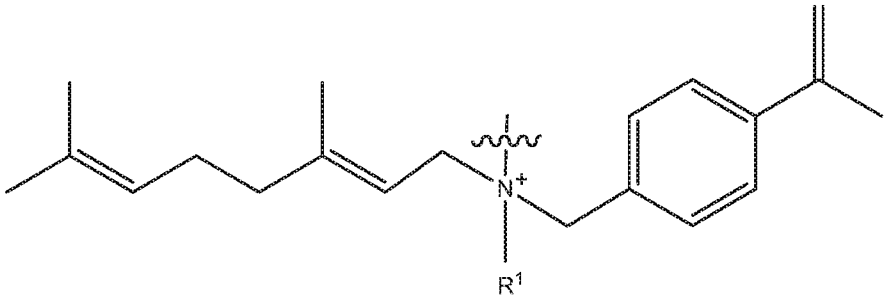

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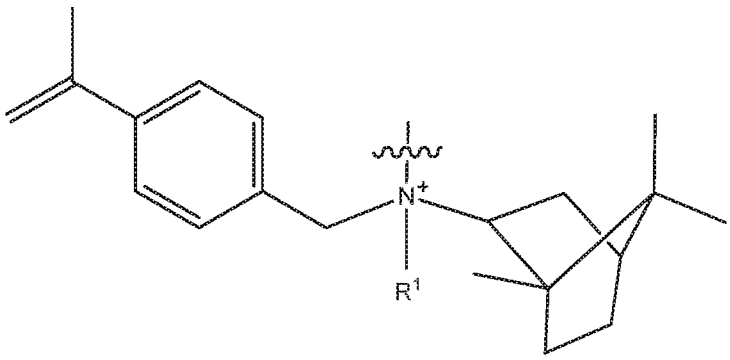

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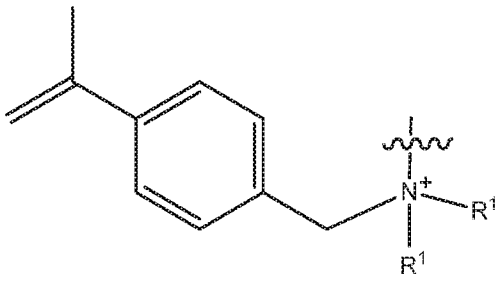

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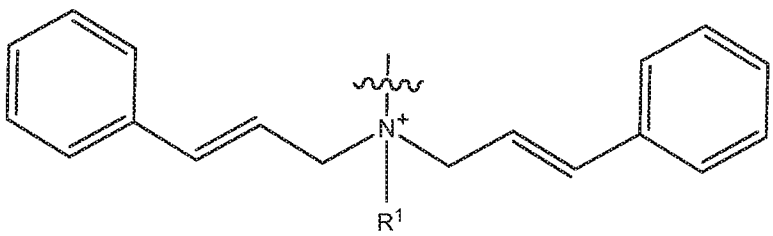

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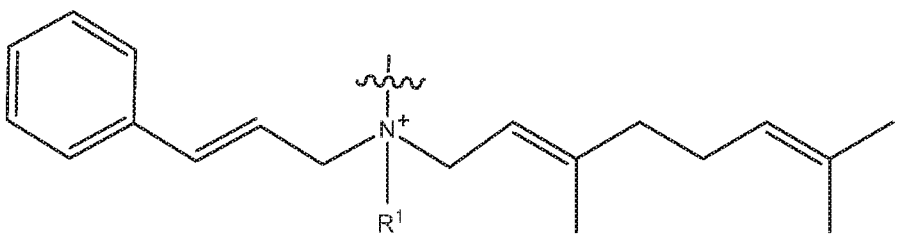

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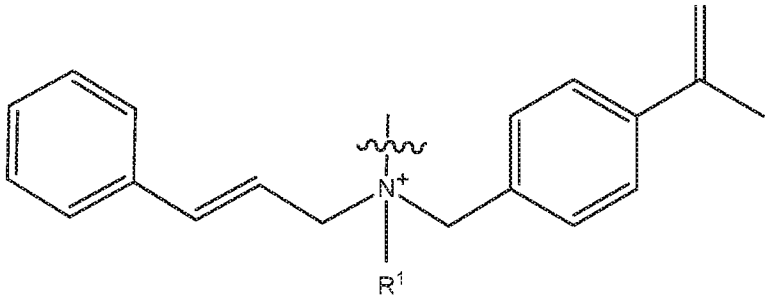

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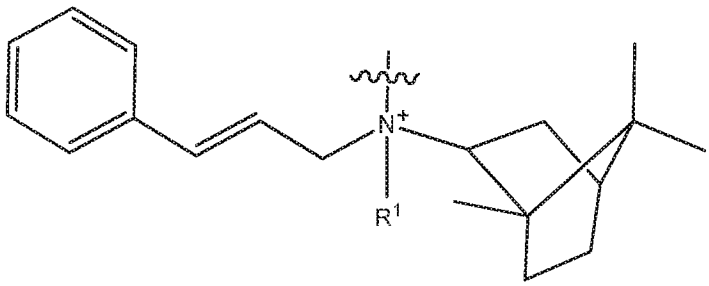

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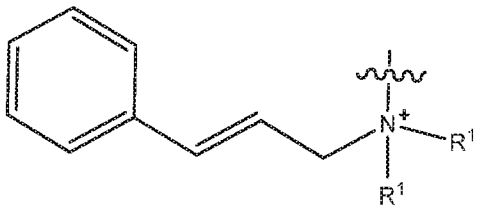

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wherein R^1 is an alkyl group having from 1 to 4 carbon atoms.

The particle of the present invention may be in the form of a tertiary amine, or in the form of a protonated ammonium salt of said tertiary amine, or in the form of a quaternary ammonium salt, as described hereinabove. Since an ammonium group is positively charged, its charge should be balanced with an anion. Preferably, in a particle according to the invention this anion is a halide, e.g. fluoride, chloride, bromide or iodide, and fluoride is most preferred. Other possible anions include, but are not limited to, bicarbonate, nitrate, phosphate, acetate, fumarate, succinate and sulfate. Each possibility represents a separate embodiment of the present invention.

Anti-microbially active amines comprising quaternary ammonium groups with one long alkyl group

In accordance with another embodiment, the anti-microbially active group of the present invention is a quaternary ammonium group, the nitrogen atom of each quaternary ammonium group having one bond to an alkyl group having from 4 to 18

carbon atoms, and a remainder of bonds each being to an alkyl group having from 1 to 3 carbon atoms.

Since an ammonium group is positively charged, its charge should be balanced with an anion. Any of the counter-ions described above may be used to counter-balance the quaternary ammonium group.

In some embodiments, the nitrogen atom of each quaternary ammonium group has (i) at least one bond to the inorganic core; (ii) one bond to the alkyl group having from 4 to 18 carbon atoms, and (iii) the remainder of the bonds each being to an alkyl group having from 1 to 3 carbon atoms.

The term "quaternary ammonium group" refers to a group of atoms consisting of a nitrogen atom with four alkyl groups attached thereto, wherein each of the alkyl groups is attached to the nitrogen through a carbon atom. The term "long alkyl group" or chain refers to such an alkyl group or chain which is substituted on the nitrogen atom of the quaternary ammonium group and which has between 4 and 18 carbon atoms. In some currently preferred embodiments, the alkyl group is an alkyl group having 4 to 10 carbon atoms. In other currently preferred embodiments, the alkyl group is an alkyl group having 6, 7, or 8 carbon atoms, with each possibility representing a separate embodiment of the present invention.

In other currently preferred embodiments, the alkyl group having from 1 to 3 carbon atoms is a methyl group.

Organic polymeric Cores

In some embodiments, the core of the particles is an organic polymeric core. In one embodiment, the organic core comprises at least one aliphatic polymer. An "aliphatic polymer" as used within the scope of the present invention refers to a polymer made of aliphatic monomers that may be substituted with various side groups, including (but not restricted to) aromatic side groups. Aliphatic polymers that may be included in particles according to the present invention comprise nitrogen atoms (as well as other heteroatoms) as part of the polymeric backbone. Non-limiting examples of aliphatic polymers are polyethylene imine (PEI), polyvinyl amine (PVA), poly(allyl amine) (PAA), poly(aminoethyl acrylate), polypeptides with pending alkyl-amino groups, and chitosan. Each possibility represents a separate embodiment of the present

invention. In one currently preferred embodiment, the polymer is polyethylene imine (PEI).

In another embodiment, the organic core comprises at least one aromatic polymer selected from the following group: aminomethylated styrene polymers, aromatic polyesters, preferably polyethylene terephthalate, and polyvinyl pyridine. The polymeric core may be linked to the anti-microbially active group directly or through a linker. Each possibility represents a separate embodiment of the present invention.

In some embodiments, the anti-microbially active group is linked to the polymeric core through a linker. In these embodiments, the linker may be selected from:

(a) a C1 to C17 alkylene substituted with at least one carboxyl moiety and at least one amino moiety, wherein the carboxyl end is attached to the polymer core and the amino end is attached to the antibacterial functional group (or is a part of the antibacterial functional group). This linker may derived from an amino acid of natural or synthetic source having a chain length of between 2 and 18 carbon atoms, or an acyl halide of said amino acid. Non-limiting examples for such amino acids are 18-amino octadecanoic acid and 18-amino stearic acid;

(b) a C1 to C18 alkylene, preferably a C2 to C18 alkylene. This linker may be derived from a di-halo alkylene, which is functionalized at each end with the core and anti-microbially active group, respectively, by replacement of the halogen moiety; and

(c) aromatic molecules derived from 4,4-biphenol, dibenzoic acid, dibenzoic halides, dibenzoic sulphonates, terephthalic acid, tetrphthalic halides, and terephthalic sulphonates. This linker is functionalized with the core and anti-microbially active group, respectively, through the functional group thereof (i.e., hydroxyl, carboxy or sulfonate).

Each possibility represents a separate embodiment of the present invention.

Various polymeric chains may provide a range of properties that themselves may be an accumulation of the various polymer properties, and may even provide unexpected synergistic properties. Examples of such mixed polyamine nanoparticles include: crosslinking of aliphatic and aromatic polyamines such as polyethyleneimine and poly(4-vinyl pyridine) via a dihaloalkane; mixture of linear short chain and

branched high molecular weight polyethyleneimines; interpenetrating compositions of polyamine within a polyamine scaffold such as polyethyleneimine embedded within crosslinked polyvinyl pyridine nanoparticles, or even interpenetrating a polyamine into a low density non-amine scaffold such as polystyrene nanoparticles. In other words, the use of polyamine combinations for the purpose of forming nanoparticles, either by chemical crosslinking or physical crosslinking (interpenetrating networks) may afford structures of varying properties (such as being able to better kill one bacteria vs. another type of bacteria). Such properties may be additive or synergistic in nature.

In one specific embodiment, the polymeric core is cross-linked with a cross-linking agent. The preferred degree of cross-linking is from 1% to 20%, when crosslinking of from about 2% to about 5% is preferable. The crosslinking may prevent unfolding of the polymer and separation of the various polymeric chains that form the particle.

Crosslinking, as may be known to a person skilled in the art of organic synthesis and polymer science, may be affected by various agents and reactions that are *per se* known in the art. For example, crosslinking may be affected by alkylating the polymer chains with dihaloalkane such as dibromoethane, dibromocyclohexane, or bis-bromomethylbenzene. Alternatively, crosslinking by reductive amination may be used. In this method a polyamine with primary amines is reacted with a diketone or with an alkane dialdehyde to form an imine crosslinker which is then farther hydrogenated to the corresponding amine. This amine may be further reacted to form an antimicrobial effective quaternary ammonium group. In such a method, instead of dihaloalkanes or dialdehydes one may use a tri or polyhaloalkanes or polyaldehydes or polyketones.

The preferred polymers useful for making particles according to the invention are those having chains made of 30 monomer units, preferably 100 monomer units that may be crosslinked using less than 10% of crosslinking agent. The longer the polymers are, the fewer crosslinking bonds are needed to afford an insoluble nanoparticle. Branched polymers are preferred for crosslinking as small amount of crosslinking is required to form insoluble nanoparticles.

In some embodiments, at least about 10% of the amine groups in the organic polymeric core are the anti-microbially active tertiary amine or quaternary ammonium groups or salts thereof, as described herein.

In a preferred embodiment, the polymeric particles according to the invention have functional groups that are capable of reacting with a host polymer or with monomers thereof. Such functional groups are designed to allow the particles to be bound chemically to a hosting matrix.

Inorganic cores

In some embodiments, the core of the particles of the present invention is an inorganic core comprising one or more inorganic materials. Inorganic cores have a few advantages over organic polymeric cores: 1) higher stability at elevated temperature; 2) higher chemical stability towards various solvent and reagents; 3) improved mechanical strength; 4) better handling qualities in matrices due to their amphipathic nature; and 5) lower cost.

An additional advantage of inorganic cores relates to the insertion of the functionalized particles into a polymeric matrix. In the case where matrix polymerization involves radical polymerization (*e.g.* acrylate resins), inorganic cores do not interfere with the polymerization process and hence do not jeopardize the mechanical properties of the finalized substrate, as opposed to polymeric cores which tend to interfere with the polymerization reaction.

In one embodiment, the core of the particles of the present invention comprises silica (silicon dioxide, or SiO_2). The silica may be in any form known in the art, non-limiting examples of which include amorphous silica, dense silica, aerogel silica, porous silica, mesoporous silica and fumed silica.

In another embodiment, the core of the particles of the present invention comprises glasses or ceramics of silicate (SiO_4^{-4}). Non-limiting examples of silicates include aluminosilicate, borosilicate, barium silicate, barium borosilicate and strontium borosilicate.

In another embodiment, the core of the particles of the present invention comprises surface activated metals selected from the group of: silver, gold, platinum, palladium, copper, zinc and iron.

In another embodiment, the core of the particles of the present invention comprises metal oxides selected from the group of: zirconium dioxide, titanium dioxide, vanadium dioxide, zinc oxide, copper oxide and magnetite.

The inorganic core typically has a solid uniform morphology with low porosity or a porous morphology having pore size diameter of between about 1 to about 30 nm.

Depending on the chemical nature of the inorganic core, the core may be attached to the anti-microbially active group directly or through a linker. Preferably a silica (SiO_2) based inorganic core may be attached to the anti-microbially active group through a linker, while silicates (SiO_4^{-4}), metals or metal oxides may be attached to the anti-microbially active group directly or through a linker.

In some embodiments, the inorganic core is directly attached to the anti-microbially active group. In other embodiments, the inorganic core is attached to the anti-microbially active group through a linker. In some embodiments, the linker is selected from the following groups: a C1 to C18 alkylene substituted with at least one silane moiety; a C1 to C18 alkylene substituted with at least one phosphate moiety; a C1 to C18 alkylene substituted with at least one anhydride moiety; a C1 to C18 alkylene substituted with at least one carboxylate moiety; and a C1 to C18 alkylene substituted with at least one glycidyl moiety. In some currently preferred embodiments, the linker is selected from the following groups: a C3 to C18 alkylene substituted with at least one silane moiety; a C3 to C18 alkylene substituted with at least one phosphate moiety; a C3 to C18 alkylene substituted with at least one anhydride moiety; a C3 to C18 alkylene substituted with at least one carboxylate moiety; and a C3 to C18 alkylene substituted with at least one glycidyl moiety. Each possibility represents a separate embodiment of the present invention.

The inorganic core of the particle as described above may generally be in a form selected from a sphere, amorphous polygonal, shallow flake-like and a rod. In some representative embodiments, the inorganic core is spherical and has a diameter between about 5 to about 100,000 nm with pore diameter of about 1 to about 30 nm. In another embodiment, the inorganic particle is in a form of a rod, having a diameter of

between about 10 to about 1,000 nm and length between about 10 to about 1,000,000 nm and a pore diameter of about 1 to about 30 nm. Each possibility represents a separate embodiment of the present invention.

Nanoparticles embedded in a hosting matrix

According to another aspect, the present invention provides a composition having a liquid or solid matrix embedding a plurality of particles as described above, wherein the particles are embedded in the matrix through covalent or non-covalent interactions.

The matrix is preferably a polymeric matrix comprising a thermoplastic polymer selected from the group consisting of polyethylene, polypropylene, silicone, epoxy resin, composite materials and acrylic polymers such as poly methyl methacrylate.

Other types of substances that may serve as hosts are ceramics, composite materials of polymeric material and inorganic solids, plant powders and particles compressed into a solid article, and organic and inorganic glues. Other substances may be selected from metal coatings and other solid, semisolid or gel-like materials.

Another polymer matrix to be used in the context of the present invention is resins used in dental and orthopedic composite materials. In such applications, antibacterial particles could be first dispersed within the resin part or added simultaneously with filler or any other solid ingredients (if any). Most of these resins are acrylic or epoxy type monomers that undergo polymerization in-vivo.

In some embodiments, embedding functionalized particles into polymeric matrices may be achieved by a variety of methodologies. For example, embedding functionalized microparticles into a polypropylene host matrix was obtained by two methodologies: A) Extrusion technology: the particles were added into molten polymer, preferably into twin-coned extruder. B) Polypropylene was heated in xylene, toluene or their derivatives under reflux conditions to achieve the complete dissolution of the polymer. The antibacterial particles were then dispersed in the same solvent as used for the polymer and the mixture was added to the dissolved polymer using overhead stirrer or homogenizer. After complete dispersion of particles within

the polymer, the solvent was evaporated using conventional distillation or evaporation methods.

Thus, according to some embodiments, the present invention provides a method for preparing a composition comprising embedding a plurality of particles as described above, wherein the particles are embedded in the matrix, the method comprising step of adding the particles as described above, into a molten polymer matrix utilizing extrusion.

The embedment of anti-bacterial particles is mainly due to mechanical forces. These particles are "locked" between the polymer chains in a three-dimensional matrix, preventing them from migrating out from the complex network. The strong hydrophobic nature of these particles also plays a role in preventing the particles from moving into the hydrophilic surrounds such as in the case of dental, orthopedic or other medical and dental applications.

In some embodiments, particles according to the invention are homogeneously distributed on the outer surface of the matrix in a surface concentration of between about 1 to about 100 particles per sq. μm. The term "homogeneous distribution" is used to denote a distribution, characterized in that the standard deviation of the number of particles per sq. um is no more than the average number of particles per sq. um. A homogeneous distribution is preferred for reproducibility and product specifications. If the distribution is not even, the product may exhibit different properties at different areas. The distribution of the particles away from the outer surface, that is, their bulk concentration, may be similar to that on the outer surface. As a general rule, the total surface of the particles preferably occupies at most about 20% of the surface of the matrix, preferably between 1% to 15%, more preferably between 1% and 5% and most about between 1% and 3% of the surface of the matrix.

According to some embodiments, on the average, every sq. μm of outer surface of matrix will have at least one portion with surface concentration of 1 anti-microbial active group per sq. nm, and that the size of such portion will be at least 100 nm^2.

The polymeric particles may be physically entrapped within the matrix, chemically bound thereto, or both. In case the particles are to be chemically bound to the host, the particles have functional groups that are capable of reacting with the host

matrix (e.g., host polymer, or with monomers thereof. Thus, in some embodiments, the particles of the present invention have functional groups that are capable of reacting with a host polymer or matrix. Such functional groups are designed to allow the particles to be chemically bound to the hosting matrix.

Polymeric particles of the present invention may also include tertiary amines or quaternary ammonium groups that are not anti-microbially active. However, the more anti-microbially active groups there are, the more preferred is the polymer, and a particle including an organic core according to the invention is characterized by having at least one anti-microbially active group per 10 sq. nm.

Preparation of Particles

The particles of the present invention may be prepared in accordance with a variety of processes, depending on the nature of the core, the anti-microbially active group, and the presence or absence of linkers. Some non-limiting examples of preparation methods are provided below.

Particles comprising (i) an organic polymer core; and (ii) anti-microbially active groups selected from the group consisting of (a) a tertiary amine comprising at least one terpenoid moiety and optionally an alkyl group having from 1 to 4 carbon atoms, or a salt of said amine; and (b) a quaternary ammonium group comprising at least one terpenoid moiety and optionally one or more alkyl groups having from 1 to 4 carbon atoms, may be prepared by functionalizing the polymeric core with said tertiary amine or said quaternary ammonium group as described above.

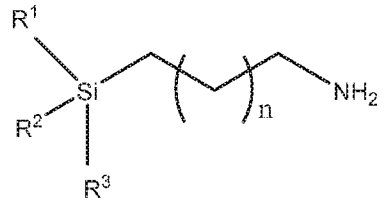
Particles comprising (i) an inorganic polymer core; and (ii) anti-microbially active groups selected from the group consisting of (a) a tertiary amine comprising at least one terpenoid moiety and optionally an alkyl group having from 1 to 4 carbon atoms, or a salt of said amine; and (b) a quaternary ammonium group comprising at least one terpenoid moiety and optionally one or more alkyl groups having from 1 to 4 carbon atoms, may be prepared by reacting the inorganic core with a linker moiety to create a surface functionalized core; and functionalizing the obtained product to generate a tertiary amine or said quaternary ammonium group as described above.

Particles comprising (i) an inorganic core; and (ii) anti-microbially active groups comprising a quaternary ammonium group chemically bound to one alkyl group having from 4 to 18 carbon atoms and a reminder of bonds each being to an

alkyl group having from 1 to 3 carbon atoms, may be prepared by (i) reacting said inorganic core with a linker moiety to create a primary amine surface functionalized core; and (ii) functionalizing the product of step (a) to generate a quaternary ammonium group.

Alternatively, particle comprising (i) an inorganic core; and (ii) anti-microbially active groups comprising a quaternary ammonium group chemically bound to one alkyl group having from 4 to 18 carbon atoms and a reminder of bonds each being to an alkyl group having from 1 to 3 carbon atoms, may be prepared by (i) reacting the inorganic core with a linker moiety comprising of a leaving group selected from ethoxy, methoxy, sulfonate and halide; and (ii) functionalizing the product of step (a) to generate a quaternary ammonium group as described above.

As contemplated herein, inorganic core – linker – anti-microbially active group adduct may be formed using a variety of reagents. The choice of the reagent depends on the nature of the anti-microbially active group. For example, when the anti-microbially active group is a tertiary amine or a quaternary ammonium group comprising at least one terpenoid moiety, a preferred reagent for coupling the inorganic core to the anti-microbially active group is represented by the structure of formula (I):



(I)

wherein

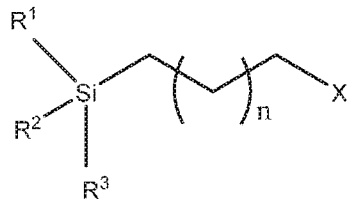
R^1, R^2 and R^3 are independently selected from the group consisting of ethoxy, methoxy, methyl, ethyl, hydrogen, sulfonate and halide, wherein at least one of R^1, R^2 and R^3 is selected from ethoxy, methoxy, sulfonate (e.g., mesyl, tosyl) and halide; and

n is an integer between 1 and 16;

wherein the reagent is capable of being chemically bound to the surface of the inorganic core through the silicon atom, and wherein the anti-microbially active group is introduced by functionalizing the primary amine to obtain an anti-microbially active

tertiary amine or quaternary ammonium group containing at least one terpenoid group, as described above.

Alternatively, when the anti-microbially active group is a quaternary ammonium group containing one alkyl group having 4 to 18 carbon atoms, a preferred reagent for coupling the inorganic core to the anti-microbially active group is represented by the structure of formula (II):



(II)

wherein

R^1, R^2 and R^3 are independently selected from the group consisting of ethoxy, methoxy, methyl, ethyl, hydrogen, sulfonate and halide, wherein at least one of R^1, R^2 and R^3 is selected from ethoxy, methoxy, sulfonate (e.g., mesyl, tosyl) and halide;

X is selected from the group consisting of NH_2, halide, sulfonate and hydroxyl; and

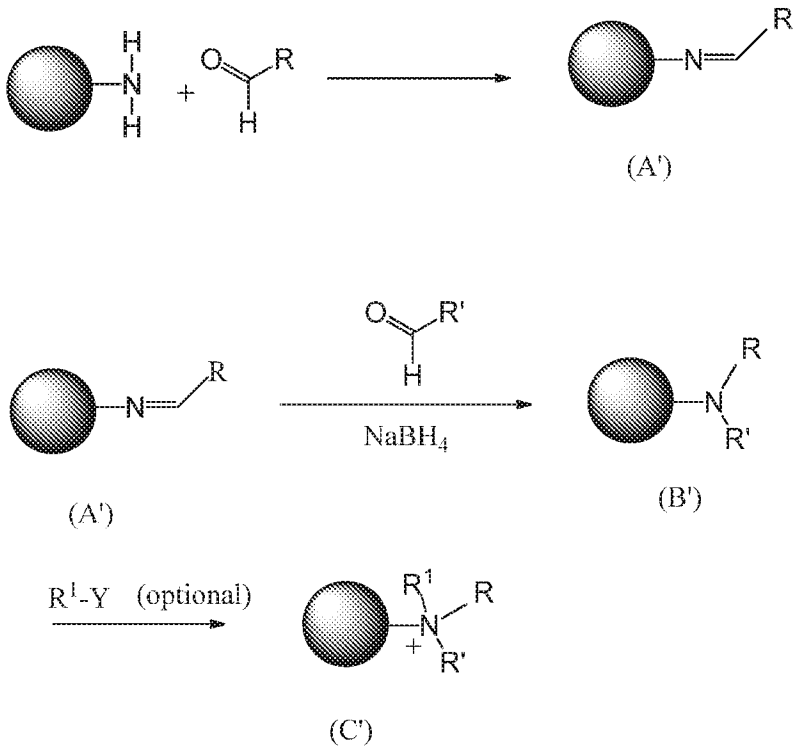
n is an integer between 1 and 16;

wherein said reagent is capable of being chemically bound to the surface of said inorganic core through the silicon atom, and wherein the anti- microbially active group is introduced by substituting the group X with an anti-microbially active group, or converting the group X to an anti-microbially active group.

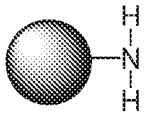
It will be apparent to a person of skill in the art that other linker moieties/reagents may be used, depending on the desired linker group. A person of skill in the art will know to design reagents and reactions to prepare other linkers contemplated by the present invention, e.g., a C1 to C18 alkylene substituted with at least one phosphate moiety; a C1 to C18 alkylene substituted with at least one anhydride moiety; a C1 to C18 alkylene substituted with at least one carboxylate moiety; and a C1 to C18 alkylene substituted with at least one glycidyl moiety. In currently preferred embodiments, the linker is a C3 to C18 alkylene substituted with at least one phosphate moiety; a C3 to C18 alkylene substituted with at least one

anhydride moiety; a C3 to C18 alkylene substituted with at least one carboxylate moiety; and a C3 to C18 alkylene substituted with at least one glycidyl moiety.

A representative method for preparing particles according to the present invention wherein the anti-microbially active group is a tertiary amine or a quaternary ammonium group comprising at least one terpenoid moiety is represented in Scheme 1. In accordance with Scheme 1, a core as defined herein is functionalized with a primary amine. The primary amine reacts with an aldehyde to yield initially an imine (Schiff base) intermediate of formula (A'), which is then reacted with a second aldehyde under reductive amination conditions to yield a tertiary amine of formula (B'). RC(=O)H and R'C(=O)H each represent an aldehyde which is a terpenoid or which is derived from a terpenoid. RC(=O)H and R'C(=O)H may be the same or different from each other. Conversion of the tertiary amine to the quaternary ammonium group is optional, and involves reaction of the tertiary amine with a group R^1-Y wherein R^1 is a C_1-C_4 alkyl and Y is a leaving group such as halogen or sulfonate.



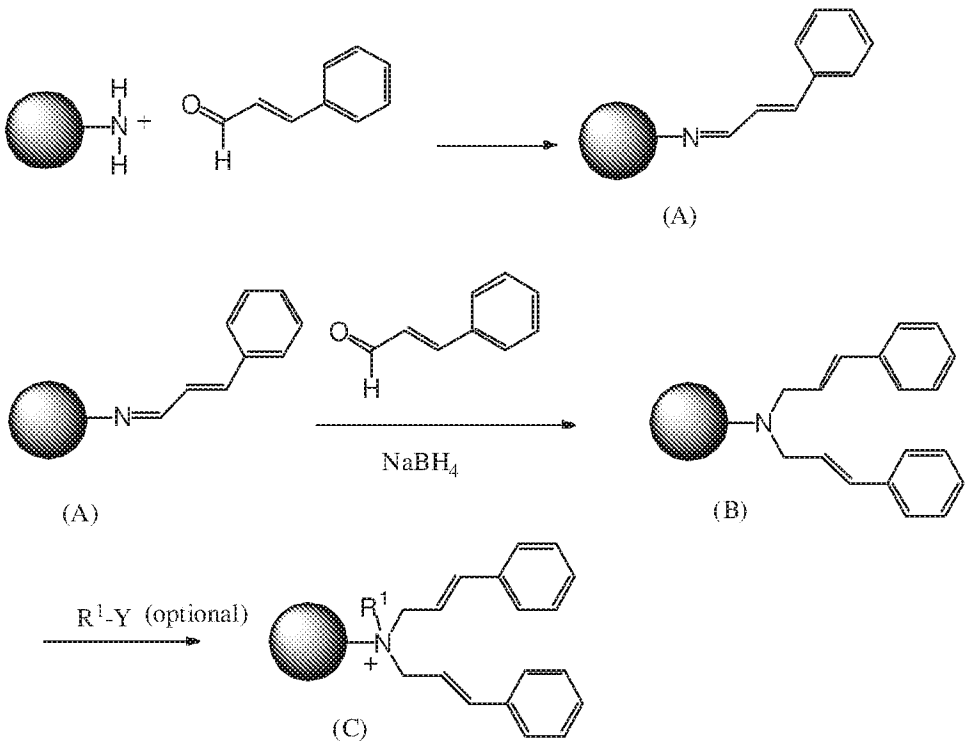
Scheme 1.

It is understood that that the group  may represents any one or more of the following:

1. An organic core directly bound to NH_2.
2. An organic core bound to NH_2 through any linker as described herein.
3. An inorganic core directly bound to NH_2.
4. An inorganic core bound to NH_2 through any linker as described herein.

The exemplified reaction may be a "one pot synthesis", or it may include two sequential reactions with isolation of an intermediate formed in the first step. The first step is the formation of intermediate (A'), which is an imine (Schiff base), by reacting an amine functionalized core with a terpenoid moiety in the presence of a reducing agent, in this case cinnamyl in the presence of $NaBH_4$. The imine functionalized core can be isolated at this stage if desired. Alternatively, further reacting intermediate (A') with a terpenoid moiety in the presence of a reducing agent yields a tertiary amine comprising two terpenoid moieties (B'). In order to obtain the quaternary ammonium, additional alkylation step is performed as described in Scheme 1.

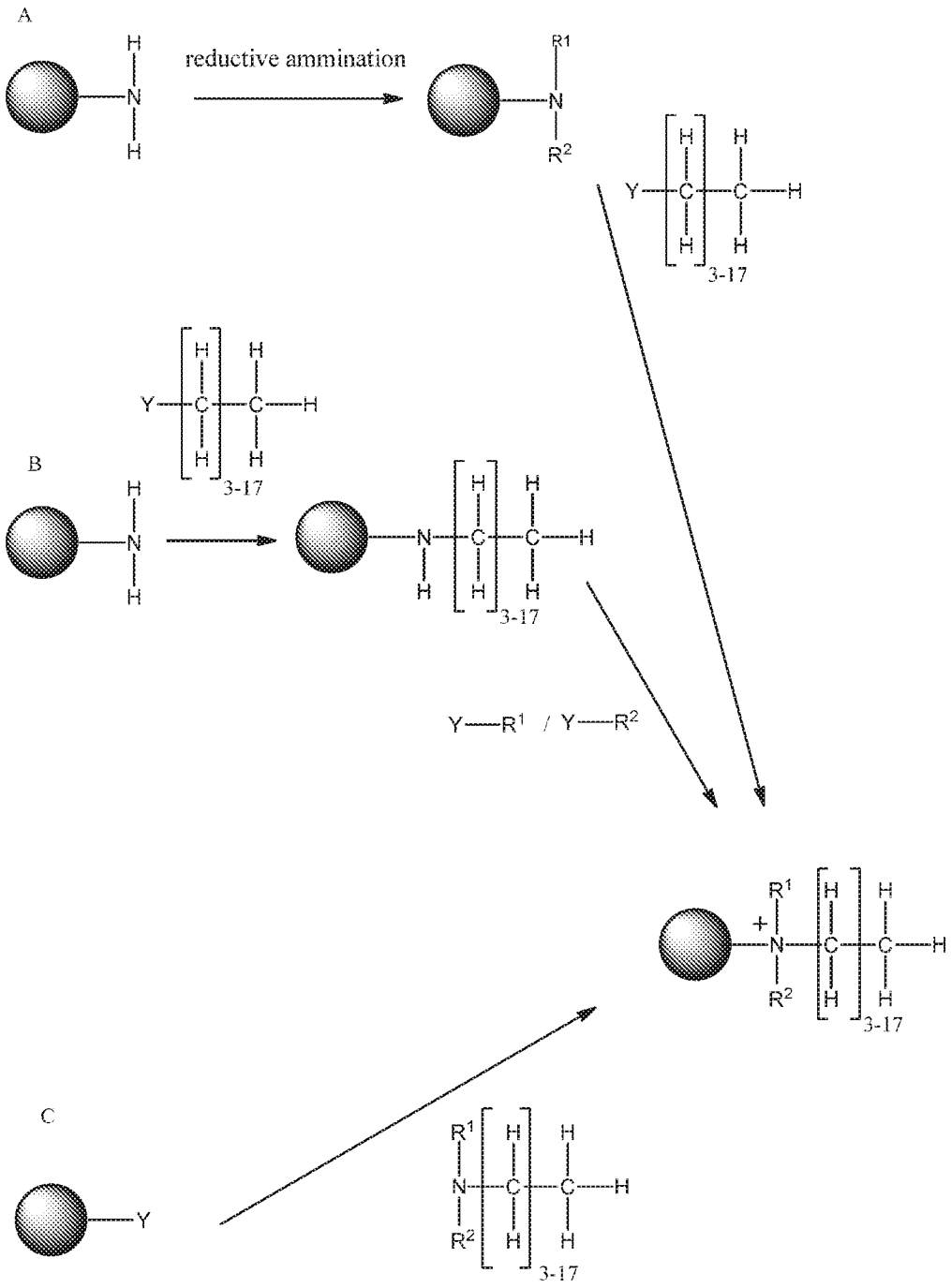
This process is exemplified in Scheme 2 for cinnamaldehyde, but is applicable to other aldehydes.



Scheme 2: Representative scheme of preparation of cinnamyl adduct product with core particle via amino-functional linker. Conversion of the tertiary amine to the quaternary ammonium group is optional, and involves reaction of the tertiary amine with a group R^1-Y wherein R^1 and Y are as defined above.

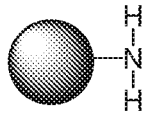
The imine particle which is an intermediate in the process for preparing the anti-microbially active particles, is new, and represents a separate embodiment of the present invention. Thus, in some embodiments, the present invention provides a particle comprising (i) an inorganic core or an organic polymeric core; and (ii) an imine moiety chemically bound to the core, preferably at a surface density of at least one imine group per 10 sq. nm, wherein the imine group comprises a terpenoid moiety. The imine moiety is generally represented by the structure of formula (B') in Scheme 1. A more specific embodiment is the structure of formula (B) in Scheme 2. It is understood by a person of skill in the art that other imine intermediate compounds comprising other terpenoids groups as described herein, are also encompassed by the present invention.

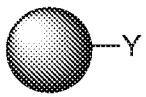
A representative method for preparing particles according to the present invention wherein the anti-microbially active group is a quaternary ammonium group containing one alkyl group having 4 to 18 carbon atoms is presented in Scheme 3:



Scheme 3: schematic illustrations of three pathways to prepare quaternary ammonium salts (QAS) functionalized particle. A) by first utilizing reductive

amination to achieve tertiary amine, followed by an alkylation reaction, B) by stepwise alkylation reactions; and C) by reacting a linker functionalized with a leaving group (e.g., Cl or other halogen) with tertiary amine. R^1 and R^2 represent C_1-C_3 alkyls such as methyl, ethyl, propyl or isopropyl. R^1 and R^2 may be different or the same group. Y represents any leaving group, for example Cl, Br or I, or a sulfonate (e.g., mesyl, tosyl).

It is understood that that the group  has any one of the meanings as described above for Schemes 1 and 2.

It is understood that that the group  may represents any one or more of the following:

1. An organic core directly bound to Y.
2. An organic core bound to Y through any linker as described herein.
3. An inorganic core directly bound to Y.
4. An inorganic core bound to Y through any linker as described herein.

Preparation of Core Particles

Porous silica materials can be prepared by reaction of $SiCl_2$ with alcohol or water, followed by drying using centrifugation and/or heating utilizing airflow or under vacuum conditions. Dense fumed silica particles (pyrogenic) were prepared by pyrolysis of $SiCl_4$.

An alternative preparation method of silica core material can be carried by the hydrolysis of tetraethylorthosilicate (TEOS) or tetramethyl orthosilicate (TMS) in the presence of alcohol or water solution and under basic (Stober) or acidic catalytic conditions.

Mesoporous silica particles can be prepared by hydrolysis of TEOS or TMS at low temperatures, preferably in a temperature not exceeding 60 °C, followed by dehydration by centrifugation and/or evaporation under airflow or vacuum conditions.

Dense particles can be prepared utilizing intense heating in a process called calcination. Typically, such process takes place at high temperatures at about 250 °C.

Core preparation and functionalization can occur by a solid support method, or a solution method (Scheme 4).

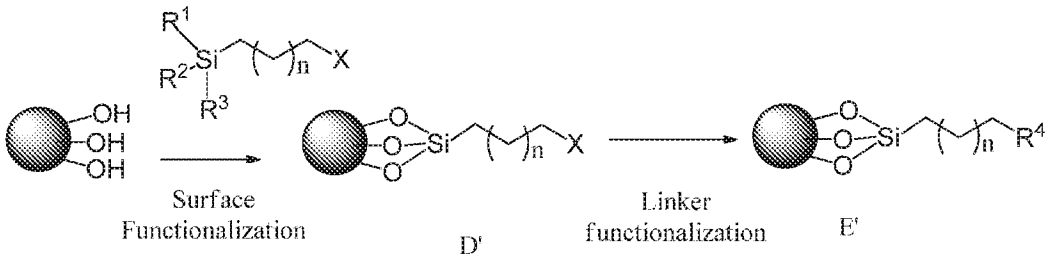
Solid support method

Preparation of functionalized particles is conducted in two general steps. First, the linker molecule is allowed to condense onto particles surface (surface functionalization) via hydrolysis of leaving groups to give an intermediate of formula (D'). Second, functional sites of the linker molecule undergo further functionalization (linker functionalization) as mentioned in any ones of Schemes 1-3 to give a functionalized particle of formula (E').

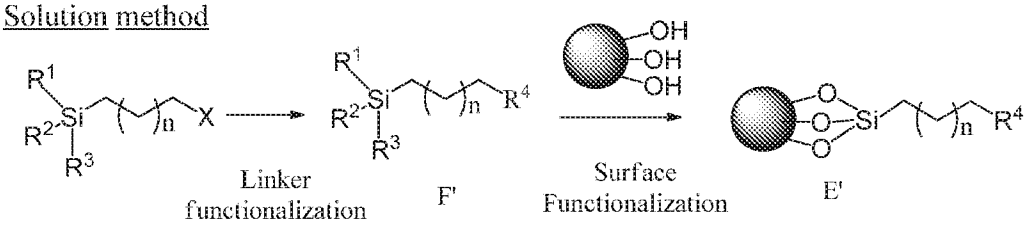
Solution method

In this method, the linker molecule is first functionalized with antimicrobial active group to give an intermediate of formula (F').In the second stage intermediate (F') is allowed to settle onto particle's solid surface (surface functionalization) to give a functionalized particle of formula (E').

Solid support method



Solution method



Scheme 4. Schemes of solid support and solution methods of particles functionalization.

R^1, R^2 and R^3 are independently selected from the group consisting of ethoxy, methoxy, methyl, ethyl, hydrogen, sulfonate and halide, wherein at least one of R^1, R^2 and R^3 is a leaving group selected from ethoxy, methoxy, sulfonate (e.g., mesyl, tosyl) and halide. For the sake of clarity the scheme presents a case where R^1, R^2 and R^3 represent leaving groups.

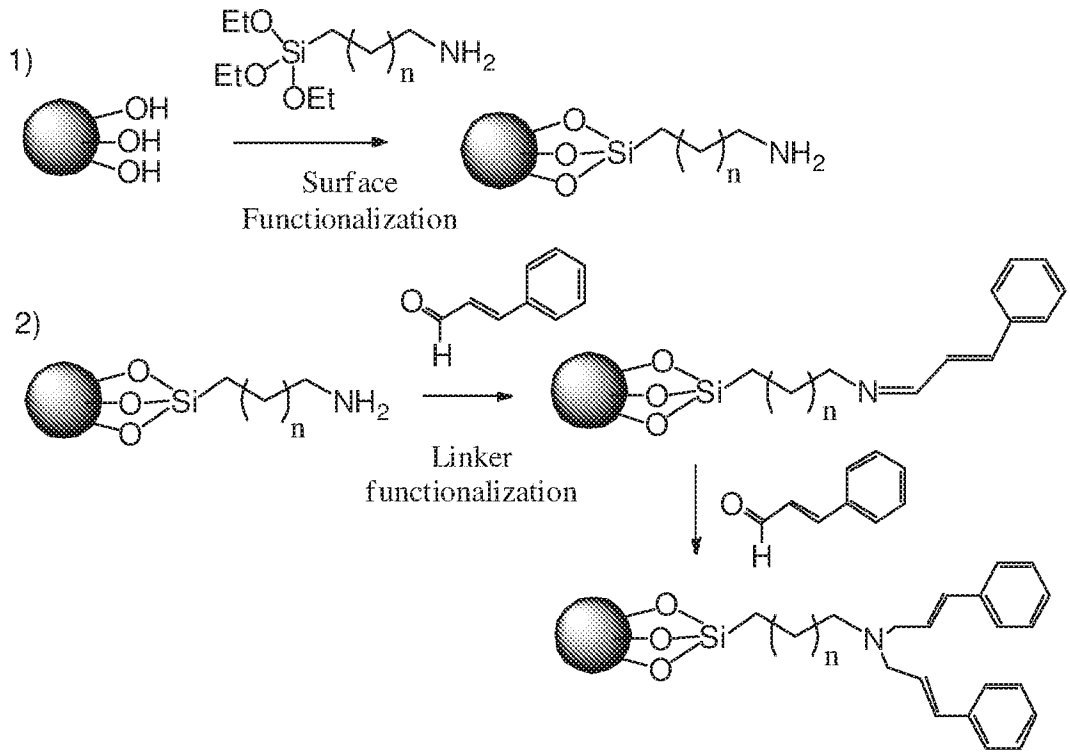
R^4 represents an anti-microbial group as described herein;

X is from the group consisting of NH_2, halide, sulfonate and hydroxyl; and

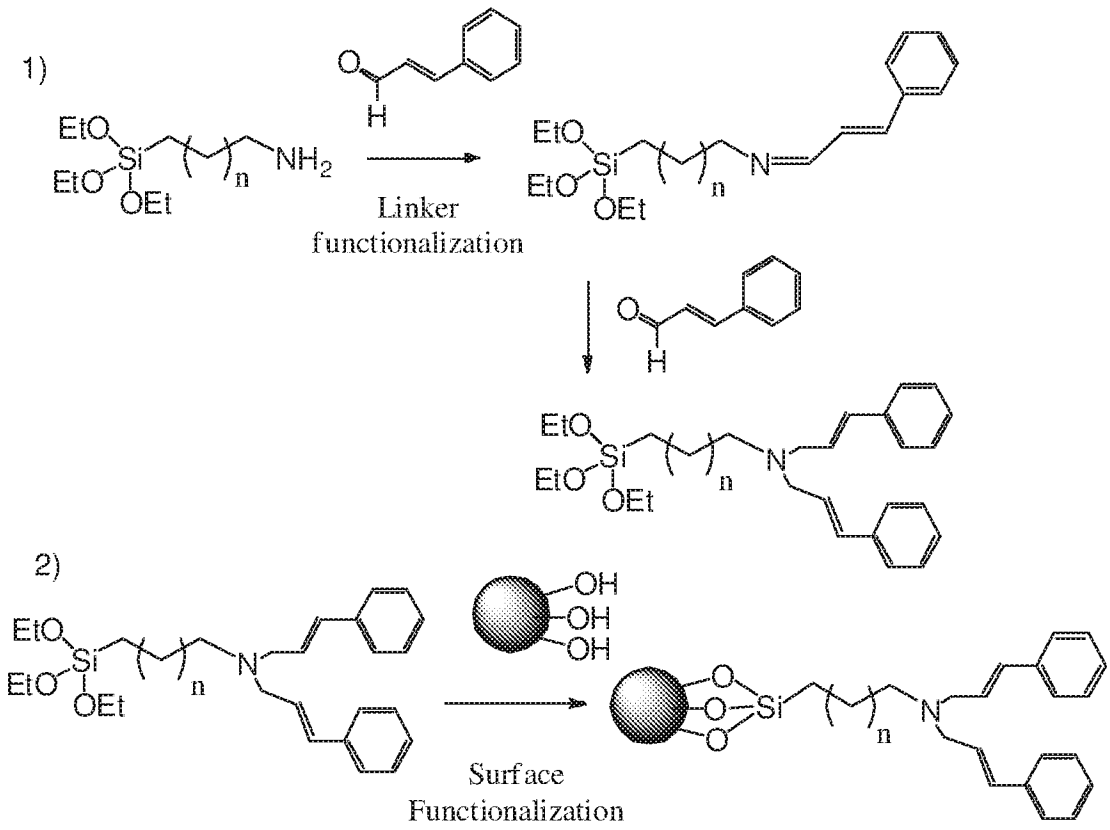
n is an integer between 1 and 16.

This process is exemplified in Scheme 5 for cinnamaldehyde, but is applicable to other aldehydes.

Solid support method



Solution method



Scheme 5: Representative scheme of preparation of di-cinnamyl adduct product with core particle functionalized utilizing a linker comprising triethoxysilyl moiety and an amine moiety, by both solid support method and solution method. n is an integer of 1 to 16.

Methods of inhibition of bacteria

According to another aspect of the invention there is provided a method for inhibition of bacteria, by contacting the bacteria with the nanoparticle or microparticle of the present invention. The term "inhibition" is used to denote destruction, i.e. annihilation, of at least 95% of the bacteria, preferably 99%, most preferably 99.99% of the bacteria; reduction in the growth rate of the bacteria; reduction in the size of the population of the bacteria; prevention of growth of the bacteria; causing irreparable damage to the bacteria; destruction of a biofilm of such bacteria; inducing damage, short term or long term, to a part or a whole existing biofilm; preventing formation of such biofilm; inducing biofilm management; or bringing about any other type of consequence which may affect such population or biofilm and impose thereto an immediate or long term damage (partial or complete).

The term "biofilm" refers to a population of biological species (bacteria) attached to a solid surface.

The terms "anti-microbial" and "anti-bacterial" are used herein interchangeably.

In a preferred embodiment, the inhibition is achieved by contacting the bacteria with a matrix containing up to 5% w/w, more preferably up to 1% particles according to the present invention, or compositions comprising them.

Accordingly, compositions according to the invention may find utility in a broad range of applications, where decontamination or growth prevention of bacteria is required, as, for example in medicine artificial replacement of tissues such as bone, bone cements and joints (orthopedic), lenses (ophthalmology), blood vessels and stents, artificial heart valves (cardiology), artificial skin, implants (plastic surgery), intra uterin devices (gynecology), neurosurgical shunts, uretral stents coating for subcutaneous implants: insulin pumps, contraceptives, pacemakers. tubing and canulas used for intra venous infusion, tubing and canulas used for dialysis, surgical

drainage tubing, urinary catheters, endotracheal tubes, wound covering materials, sutures, catheters of all kinds that are inserted temporarily in blood vessels as well as the urinary system, shunt for use in brain applications, surgical gloves, tips for ear examination, statoscope ends and other elements used by the medical personnel; plastic wear for medical and research laboratories; food packaging, mainly for dairy products and fresh meat; paints for ships, that prevent growth of biofilm, paints for bathrooms, and many others.

One preferred use of the compositions of the present invention is in dentistry: dental adhesives, dental restorative materials such as all types of composite based materials for filling tooth-decay cavities, endodontic filling materials (cements and fillers) for filling the root canal space in root canal treatment, materials used for provisional and final tooth restorations or tooth replacement, including but not restricted to inlays, onlays, crowns, partial dentures (fixed or removable) dental implants, and permanent and temporary cements used in dentistry for various known purposes.

In one particular embodiment, the particle or composition of the present invention is intended for administration into an oral cavity. The composition may be formulated as a tooth paste, and/or may be applied to a surface or medical device selected from the group consisting of: a denture cleaner, post hygienic treatment dressing or gel, mucosal adhesive paste, a dental adhesive, a dental restorative composite based material for filling tooth, decay cavities, a dental restorative endodontic filling material for filling root canal space in root canal treatment, a dental restorative material used for provisional and final tooth restorations or tooth replacement, a dental inlay, a dental onlay, a crown, a partial denture, a complete denture, a dental implant and a dental implant abutment.

The antimicrobial property may protect the patient and the medical staff from cross contamination from patient to patient or from patient to the examiner. Self-sterilizing packaging for medicines and items that enter the operation room are also beneficial. Applications out of the medical field may for example be in athlete shoes or the inner part of a shoe wherein bacteria tend to collect, tooth brushes and any brush that comes in contact with the human body, pet cages as well as other veterinary items, etc.

The following non-limiting examples are presented in order to more fully illustrate certain embodiments of the invention. They should in no way, however, be construed as limiting the broad scope of the invention. One skilled in the art can readily devise many variations and modifications of the principles disclosed herein without departing from the scope of the invention.

EXAMPLES

Example 1: preparation of core particles of amorphous SiO_2 (silica)

Silica dioxide core particles were prepared by hydrolysis of tetraalkoxy silicate under alkaline conditions. The reaction solution was prepared by mixing ethanol, water and ammonia, keeping the pH within the range of 10-14. Controlling the particle size and the reaction rate is achieved by adjusting the concentration of water and ammonia in the reaction solution. Tetraethyl orthosilicate (TEOS) was added to the solution in one portion with stirring. The reaction mixture first turned opaque, followed by a white solid precipitation, indicating the reaction endpoint and agglomerates formation of primary particles. The particles were recovered by centrifugation filtration, rinsing in purified water and drying using freeze drying or heating. Optionally, further surface activation may be performed by shortly rinsing particles in sulfuric acid / hydrogen peroxide solution. This last step converts most of the particles' surface into hydroxyl form and promotes an efficient surface functionalization.

Example 2: Morphological characterization of silica particles

Nitrogen adsorption method was used to determine the morphology of porous silica dioxide particles by utilizing Barrett-Joyner_Halenda (BJH) model. Non-functionalized mesoporous silica dioxide particles were rinsed in Milli-Q water, dried and then degassed. Pore size was obtained from the adsorption/desorption isotherm by applying BJH model. Average particle size measured using dynamic light scattering method. Therefore, said particles are of 186 nm in diameter and having pore size of 5.0 nm.

Example 3: preparation of magnetite core particles

Magnetite (Fe_3O_4) particles were prepared by co-precipitation of Fe^{2+} and Fe^{3+} ions, from $FeCl_2$ and $FeCl_3$ in aqueous solution in basic condition utilizing NH_4OH (pH~12). After precipitation, the particles recovered under constant magnetic field. Prior to functionalization, particles were rinsed in Mili-Q water followed by vacuum drying. Surface activation of the obtained magnetite particles was performed by a short rinse of the particles in nitric acid or sulfuric acid and hydrogen peroxide solution. The last step converted most of particles' surface into hydroxy form allowing further functionalization of the core.

Example 4: Surface functionalization of inorganic core particles

Functionalization of silica particles was performed in two stages. Initially, primary amine-functionalized silica particles were prepared. The primary amine was the functionalized by reductive amination to yield a tertiary amine comprising terpenoid groups, or alternatively a quaternary ammonium group comprising one elongated alkyl chain of 8 carbons.

(a) Preparation of primary amine-functionalized silica particles

1) Dry silica particles were dispersed in 1:9 water/ethanol solution, and the pH of the mixture was adjusted to ~4.5 by the addition of glacial acetic acid. 3-aminopropyl triethoxy silane (APTS) was added to the reaction mixture in an amount that does not exceed 4 % wt/v of the total reaction mixture. The reaction was conducted at a temperature of 60° to 80 °C for about 1-3 hours. Subsequently, the amine-functionalized particles were recovered by rinsing/drying method utilizing purified water, then rinsed in alkaline solution of $NaHCO_3$, and were left to dry.

(b) Forming a tertiary amine comprising two terpenoid groups:

Tertiary amine was prepared by reacting 1° amine-functionalized particles obtained in part 1 with citral (terpenoid) at 1:10 amine to citral mole ratio and continuous reduction of imine formed in-situ by $NaBH_4$ (reductive amination). The reaction was conducted in dichloromethane at ambient conditions. Subsequently, functionalized particles were recovered by rinsing/drying method in purified water.

(c) Formation of quaternary ammonium compounds comprising elongated alkyl chain (C8):

In order to obtain the quaternary ammonium derivative, the 1° amine-functionalized particles were reacted with paraformaldehyde at 1:10 mole ratio of amine to formaldehyde unit and continuous reduction of the imine formed in-situ by $NaBH_4$ (reductive amination). The reaction was conducted in dichloromethane (DCM) for 24 hours and produced a tertiary amine intermediate. The tertiary amine was further alkylated utilizing 1.25 mole eq. of 1-iodooctane in DCM. The reaction was conducted under ambient temperature for 48 hours. Subsequently, quaternary ammonium functionalized particles were recovered by rinsing/drying method.

Example 5: Preparation and surface functionalization of organic polymeric core particles with Cinnamaldehyde

Dry polyethylene imine (PEI) was first dissolved in absolute ethanol at 1:10 wt/v ratio. 0.025 mol eq. of 1,5-diiodopentane was added to produce cross-linked PEI particles under 80°C reflux conditions for 24 hours. The particles were recovered by ethanol evaporation under heating and vacuum conditions, than re-dissolved in DCM. Functionalization was carried out by the addition of 10 mole of cinnamaldehyde to 1 mole eq. of ethylene imine unit and continuous reduction of the imine formed in-situ utilizing $NaBH_4$ (reductive amination). The reaction was conducted in DCM at ambient conditions. Subsequently, the functionalized particles recovered by rinsing/drying method in purified water.

Example 6: Anti-microbial activity of matrix comprising functionalized silica particles

Anti-microbial test conditions – direct contact test

Direct contact between bacteria and the tested materials was achieved by applying 10 µl of bacterial suspension on each tested material sample in a set of 8 wells. The plate was incubated at a vertical position for 1 hr at 37 °C. During this incubation period, the suspension's liquid evaporated and a thin layer of bacteria was obtained, ensuring direct contact between the bacteria and the tested material. The plate was then placed horizontally and 220 µl of brain-heart infusion broth were added to each well containing the material. All tests were done using Stapilococcus aureus (S. aureus) and Enterococcus faecalis (E. faecalis) as representative for Graham positive bacteria

and Pseudomonas aeruginosa (P. aeruginosa) as representative for Graham negative bacteria.

The kinetic measurement of bacterial growth was done utilizing temperature controlled microplate spectrophotometer (VERSAmax, Molecular Devices Corporation, Menlo Oaks Corporate Centre, Menlo Park, CA, USA). The microtitre plate was placed in the spectrophotometer, at 37 °C with 5 sec vortex prior to every reading. Bacterial growth was estimated by the OD changes in each well at 650 nm every 20 minutes for 24 hours.

Mixing of methyl methacrylate with functionalized particles

The functional particles were mixed with methyl methacrylate (Unifast Trad, GC America Inc) utilizing an amalgamator machine, where the particulate matter was mixed with the solid powder portion of the Unifast Trad prior to the addition of liquid monomer.

Sample preparation

1) Polypropylene comprising quaternary ammonium functionalized silica particles

Silica particles of an average diameter of 186 nm functionalized with quaternary dimethyl octyl ammonium were embedded in polypropylene. Samples of polymer films were prepared by hot molding of polypropylene and the functionalized silica particles at 0, 1 and 2 %wt/wt of particles. 5*10 mm samples of prepared films were positioned into wells of microtitre plate touching the inside sidewalls of each well.

The anti-bacterial test results demonstrated a consistently low OD level during the experiment for the polypropylene samples containing 1 and 2 %wt/wt of particles, while the polypropylene sample containing no particles and the control sample containing S. aureus demonstrated a significant OD increase (Figure 1).

Similar results were obtained in the presence of P. aeruginosa, where the polypropylene samples containing 2 %wt/wt of particles demonstrated a low OD level and the sample containing 1 %wt/wt of particles showed a slightly higher OD level. In contrast, the polypropylene sample containing no particles and the control sample containing P. aeruginosa demonstrated a significant OD increase (Figure 2).

These results reveal the anti-microbial effect obtained by the modified polypropylene substrate utilizing quaternary ammonium functionalized silica nanoparticles.

2) Poly (methyl methacrylate) comprising quaternary amine functionalized silica particles

Silica particles of an average diameter of 13 μm functionalized with quaternary dimethyl octyl ammonium were embedded in commercially available dental polymerizable methylmethacrylate (Unifast Trad, GC America inc) at concentration of 0 and 1 %wt/wt. The methylmethacrylate was mixed in a silicone crucible at a liquid/powder ratio of 2g/ml respectively, in accordance to manufacturer's instructions and then allowed to polymerize onto sidewalls of microtiter wells at 37 °C for 24 hours prior to the anti-microbial test.

The anti-bacterial test results demonstrated a consistently low OD level during the experiment for the methymethacrylate (PMMA) samples containing 1%wt/wt of particles, while the PMMA sample containing no particles and the control sample containing P. aeruginosa demonstrated a significant OD increase (Figure 3).

Similar results were obtained in the presence of S. aureus, where PMMA sample containing 1 %wt/wt of particles demonstrated a low OD level and the sample containing no particles and the control sample containing S. aureus demonstrated a significant OD increase (Figure 4).

These results reveal the anti-microbial effect obtained by the modified PMMA substrate utilizing quaternary ammonium functionalized silica macro-size particles.

3) Poly (methyl methacrylate) comprising tertiary amine functionalized silica particles

Silica particles of an average diameter of 186 nm functionalized with di-cinnamyl amine (tertiary amine) were embedded in commercially available dental polymerizable methylmethacrylate (Unifast Trad, GC America Inc.) at concentration of 0 and 1 %wt/wt. The methymethacrylate was mixed in a silicone crucible at a liquid/powder ratio of 2g/ml respectively, in accordance to manufacturer's instructions and then allowed to polymerize onto sidewalls of microtiter wells at 37 °C for 24 hours prior to the anti-microbial test.

The anti-bacterial test results demonstrated a consistently low OD level during the experiment for the methymethacrylate (PMMA) samples containing 1%wt/wt of particles, while the PMMA sample containing no particles and the control sample containing P. aeruginosa demonstrated a significant OD increase (Figure 5).

Similar results were obtained in the presence of S. aureus, where PMMA sample containing 1 %wt/wt of particles demonstrated a low OD level and the sample containing no particles and the control sample containing S. aureus demonstrated a significant OD increase (Figure 6).

These results reveal the anti-microbial effect obtained by the modified PMMA substrate utilizing di-terpenoid (tertiary amine) functionalized silica nanoparticles.

4) Poly (methyl methacrylate) comprising quaternary amine functionalized magnetite particles

Magnetite (Fe_3O_4) particles of an average diameter of 78 nm functionalized with quaternary dimethyl octyl ammonium were embedded in commercially available dental polymerizable methylmethacrylate (Unifast Trad, GC America inc) at concentration of 0, 1 and 2 %wt/wt. The PMMA was mixed in a silicone crucible at a liquid/powder ratio of 2g/ml respectively, in accordance to manufacturer's instructions and then allowed to polymerize onto sidewalls of microtiter wells at 37 °C for 24 hours prior to the anti-microbial test.

The anti-bacterial test results demonstrated a consistently low OD level during the experiment for the methymethacrylate (PMMA) samples containing 1 and 2%wt/wt of particles, while the PMMA sample containing no particles and the control sample containing E. faecalis demonstrated a significant OD increase (Figure 7).

These results reveal the anti-microbial effect obtained by the modified PMMA substrate utilizing quaternary ammonium functionalized magnetite nanoparticles.

5) Poly (methyl methacrylate) comprising quaternary amine functionalized silica particles

Silica particles of an average diameter of 186 nm functionalized with quaternary ammonium comprising di-cinnamyl methyl substitutes, were embedded in commercially available dental polymerizable methylmethacrylate (Unifast Trad) at concentration of 0, 2 and 3 %wt/wt. The PMMA was mixed in a silicone crucible at a

liquid/powder ratio of 2g/ml respectively, in accordance to manufacturer's instructions and then allowed to polymerize onto sidewalls of microtiter wells at 37 °C for 24 hours prior to the anti-microbial test. Both liquid and solid parts of the polymer material were manipulated accordingly to manufacturer's instructions and then allowed to polymerize onto sidewalls of microtiter wells at 37 °C for 24 hours prior to the anti-microbial test.

The anti-bacterial test results demonstrated a low OD level during the experiment for the methymethacrylate (PMMA) samples containing 3%wt/wt of particles, and a slightly higher level for the sample containing 2%wt/wt of particles. In contrast, the PMMA sample containing no particles and the control sample containing E. faecalis demonstrated a significant OD increase (Figure 8).

These results reveal the anti-microbial effect obtained by the modified PMMA substrate utilizing di-terpenoid quaternary ammonium functionalized silica nanoparticles.

Example 7: Mechanical tests of resins comprising functionalized particles

Poly methylmethacrylate (Unifast Trad) cylindrical specimens of 0.4 mm in diameter and 10 mm in length were prepared using polypropylene pipe-like molds. Specimens were allowed to polymerize at room temperature for 1 hour within the molds, then stored in DDW at 37 °C for 24 hours prior to testing. Each tested group contained 10 specimens of cured cement with 8 %wt/wt NPs. A control group was obtained using the polymer specimens without functionalized particles. Compressive strength test was carried out using universal testing machine (Instron 3366, Canton, MA) operated at displacement speed of 1 mm/min. Data was instantly analyzed with Merlin software which calculated the compressive strength and the Young's modulus.

The samples tested were marked as follows:

1) QASi – containing 8%wt of dimethyl octyl quaternary ammonium functionalized 186 nm silica particles.

2) QPEI - containing 8%wt of dimethyl octyl quaternary ammonium functionalized PEI particles of 24 nm.

3) A sample of unmodified poly methylmethacrylate (PMMA) resin was used as a control.

The results demonstrated relatively high stability of the modified acrylate resin comprising the silica based particles under stress conditions. The embedment of silica functionalized antibacterial particles did not jeopardize the mechanical properties of the resin, and appeared to be advantageous in terms of stress-stability in comparison to the polymeric functionalized resin (QPEI) (Figure 9B).

Example 8: antibacterial test of resins comprising functionalized particles

The samples described on Example 7 were tested for their antibacterial activity by direct contact test as described herein above (Example 6).

The results demonstrate the potent antibacterial effect of the modified resins due to the embedment of the functionalized silica-based and PEI-based particles compared with the unmodified resin control sample and the natural growth of bacteria as depicted in the presence of E. faecalis (Figure 10A) and S. aureus (Figure 10B).

Example 9: antibacterial test by imprint method

Three glass slides were coated utilizing spraying of a solution containing functionalized silica based particles onto the hydroxylated glass surface. The silane group anchored the functionalized particles to the slide upon hydrolysis of the leaving groups and the slides were further dried at elevated temperature to allow complete condensation of the particles onto to the surface. The glasses were marked as follows:

1) dimethylamine functionalized silica particles;

2) tertiary amine with two cinnamyl groups functionalized silica particles.

S. aureus suspension was applied onto each functionalized slide in a homogeneous manner. The slides were placed in contact with blood agar petri dish facing towards the agar for 15 minutes. Subsequently, the slides were removed and the petri dishes were kept in 37 °C for 24 to allow formation of colonies.

The results revealed that no colonies were formed onto the petri dish which came in contact with functionalized slide 2, demonstrating the advantageous antibacterial activity of the tertiary amine comprising two cinnamyl groups (Figure 11).

The foregoing examples of specific embodiments will so fully reveal the general nature of the invention that others can, by applying current knowledge, readily modify and/or adapt for various applications such specific embodiments without undue experimentation and without departing from the generic concept, and, therefore, such adaptations and modifications should and are intended to be comprehended within the meaning and range of equivalents of the disclosed embodiments. It is to be understood that the phraseology or terminology employed herein is for the purpose of description and not of limitation. The means, materials, and steps for carrying out various disclosed functions may take a variety of alternative forms without departing from the invention.

The scope and concept of the invention will be more readily understood by references to the claims, which follow.

CLAIMS

1. A particle comprising

(i) an inorganic core or an organic polymeric core; and

(ii) anti-microbially active groups chemically bound to the core at a surface density of at least one anti-microbially active group per 10 sq. nm,

wherein the anti-microbially active group is selected from the group consisting of (a) a tertiary amine comprising at least one terpenoid moiety and optionally an alkyl group having from 1 to 4 carbon atoms, or a salt of said amine; and (b) a quaternary ammonium group comprising at least one terpenoid moiety and optionally one or more alkyl groups having from 1 to 4 carbon atoms.

2. A particle according to claim 1, comprising:

(i) an organic polymer core; and

(ii) anti-microbially active groups chemically bound to the core at a surface density of at least one anti-microbially active group per 10 sq. nm,

wherein the anti-microbially active group is selected from the group consisting of: (a) a tertiary amine comprising at least one terpenoid moiety and optionally an alkyl group having from 1 to 4 carbon atoms, or a salt of said amine; and (b) a quaternary ammonium group comprising at least one terpenoid moiety and optionally one or more alkyl groups having from 1 to 4 carbon atoms.

3. A particle according to claim 1, comprising:

(i) an inorganic core; and

(ii) anti-microbially active groups chemically bound to the core at a surface density of at least one anti-microbially active group per 10 sq. nm,

wherein the anti-microbially active group is selected from the group consisting of: (a) a tertiary amine comprising at least one terpenoid moiety and optionally an alkyl group having from 1 to 4 carbon atoms, or a salt of said amine, wherein the terpenoid moiety is a bornyl group derived from camphor, bornyl halide or bornyl alcohol, or wherein the terpenoid moiety is derived from citral or perilaldehyde; and (b) a quaternary ammonium group

comprising at least one terpenoid moiety and optionally one or more alkyl groups having from 1 to 4 carbon atoms.

4. A particle according to claim 1, comprising:

(i) an inorganic core selected from silicate (SiO_4^{-4}), surface activated metal and metal oxide; and

(ii) anti-microbially active groups chemically bound to the core at a surface density of at least one anti-microbially active group per 10 sq. nm,

wherein the anti-microbially active group is selected from the group consisting of: (a) a tertiary amine comprising at least one terpenoid moiety and optionally an alkyl group having from 1 to 4 carbon atoms, or a salt of said amine; and (b) a quaternary ammonium group comprising at least one terpenoid moiety and optionally one or more alkyl groups having from 1 to 4 carbon atoms.

5. A particle according to claim 1, comprising:

(i) an inorganic core or an organic polymeric core; and

(ii) anti-microbially active groups chemically bound to the core at a surface density of at least one anti-microbially active group per 10 sq. nm,

wherein the anti-microbially active group is a quaternary ammonium group comprising at least one terpenoid moiety and optionally one or more alkyl groups having from 1 to 4 carbon atoms.

6. The particle of any one of the preceding claims, wherein the anti-microbially active groups are chemically bound to the core at a surface density of at least one anti-microbially active group per 1 sq. nm.

7. The particle of any one of the preceding claims, wherein the anti-microbially active group is selected from the group consisting of:

(a) a tertiary amine, the nitrogen atom of each tertiary amine comprising at least one bond to the core, one bond to a terpenoid moiety and optionally remaining bond to an alkyl group having from 1 to 4 carbon atoms or a salt of said tertiary amine;

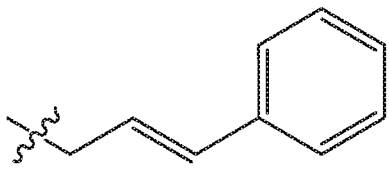
(b) a tertiary amine, the nitrogen atom of each tertiary amine comprising one bond to the core, and two bonds to terpenoid moieties which may be the same or different from each other, or a salt of said tertiary amine; and

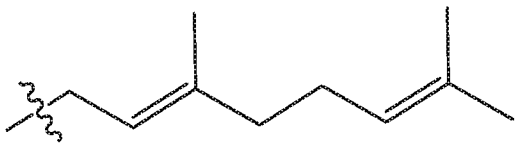
(c) a quaternary ammonium group, the nitrogen atom of each quaternary ammonium group comprising at least one bond to the core, one or two bonds to terpenoid moieties which may be the same or different from each other, and optionally remaining bonds to an alkyl group having from 1 to 4 carbon atoms.

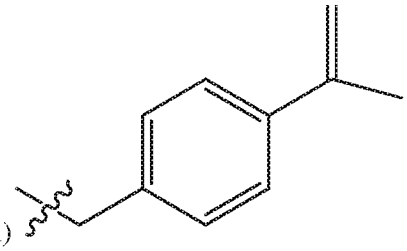
8. The particle of any one of claims 1 to 4, in the form of a protonated ammonium salt of a tertiary amine.

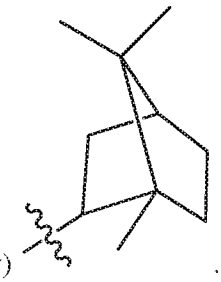
9. The particle of claim 1, wherein the at least one terpenoid moiety is a cinammyl group derived from cinnamaldehyde, cinnamic acid or cinnamyl alcohol; a bornyl group derived from camphor, bornyl halide or bornyl alcohol; a terpenoid group derived from citral; or a terpenoid group derived from perilaldehyde.

10. The particle of any one of the preceding claims, wherein the at least one anti-microbially active terpenoid moiety comprises a functional group selected from the group consisting of:

(i)  ;

(ii)  ;

(iii)  ; and

(iv)  .

11. The particle of any one of claims 1, 2 and 5, wherein said core is an organic core selected from the group consisting of:

(a) at least one aliphatic polymer selected from the group consisting of polyethylene imine (PEI), polyvinyl amine (PVA), poly(allyl amine) (PAA), poly(aminoethyl acrylate), polypeptides with pending alkyl-amino groups, and chitosan, preferably, wherein the polymer is polyethylene imine (PEI); and

(b) at least one aromatic polymer selected from the group consisting of aminomethylated styrene polymers, aromatic polyesters, preferably polyethylene terephthalate, and polyvinyl pyridine;

wherein the anti-microbially active group is attached to the organic core directly or through a linker.

12. The particle of claim 11, wherein the anti-microbially active group is attached to the organic core through a linker selected from the group consisting of:

(a) a C1 to C17 alkylene substituted with at least one carboxyl moiety and at least one amino moiety, wherein the carboxyl end is attached to the polymer core and the amino end is attached to the antibacterial functional group (or is a part of the antibacterial functional group).;

(b) a C2 to C18 alkylene; and

(c) aromatic molecules derived from 4,4-biphenol, dibenzoic acid, dibenzoic halides, dibenzoic sulphonates, terephthalic acid, tetrphthalic halides, and terephthalic sulphonates.

13. The particle of claim 11, wherein the polymeric core is cross-linked with a cross-linking agent.

14. The particle of claim 13, wherein the degree of cross-linking is from about 1% to about 20%.

15. The particle of any one of the preceding claims, wherein at least 10% of the amine groups in the polymer are the anti-microbially active tertiary amine or quaternary ammonium groups or salts thereof.

16. The particle of any one of the preceding claims, having a diameter between about 5 to about 100,000 nm, preferably, between about 10 to about 50,000 nm.

17. The particle of any one of claims 1 and 3-5, wherein said core is an inorganic core, and wherein the anti-microbially active group is attached to the inorganic core directly or through a linker.

18. The particle of claim 17, wherein said inorganic core comprises:

(a) silica (SiO_2) in a form selected from the group consisting of amorphous silica, dense silica, aerogel silica, porous silica, mesoporous silica and fumed silica;

(b) glasses or ceramics of silicate (SiO_4^{-4}) selected from the group consisting of aluminosilicate, borosilicate, barium silicate, barium borosilicate and strontium borosilicate;

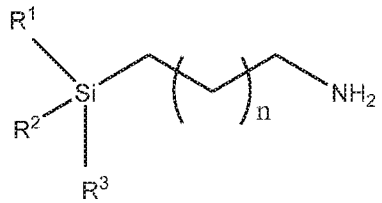
(c) surface activated metals selected from the group consisting of silver, gold, platinum, palladium, copper, zinc and iron; or

(d) metal oxides selected from the group consisting of zirconium dioxide, titanium dioxide, vanadium dioxide, zinc oxide, copper oxide and magnetite.

19. The particle of claim 17 or 18, wherein said core has a solid uniform morphology with low porosity or a porous morphology having pore size diameter of between about 1 to about 30 nm.

20. The particle of claim 17, wherein the inorganic core is attached to the anti-microbially active group through a linker, wherein the linker is selected from the group consisting of a C3 to C18 alkylene substituted with at least one silane moiety; a C3 to C18 alkylene substituted with at least one phosphate moiety; a C3 to C18 alkylene substituted with at least one anhydride moiety; a C3 to C18 alkylene substituted with at least one carboxylate moiety; and a C3 to C18 alkylene substituted with at least one glycidyl moiety.

21. The particle of claim 20, wherein the linker is a C3 to C18 alkylene substituted with at least one silane moiety, and wherein a reagent represented by the structure of formula (I) is utilized to couple the inorganic core to the anti-microbially active group:



(I)

wherein

R^1, R^2 and R^3 are independently selected from the group consisting of ethoxy, methoxy, methyl, ethyl, hydrogen, sulfonate and halide, wherein at least one of R^1, R^2 and R^3 is selected from ethoxy, methoxy, sulfonate and halide; and

n is an integer between 1 and 16;

wherein said reagent is capable of being chemically bound to the surface of said inorganic core through the silicon atom, and wherein the anti-microbially active group is introduced by functionalizing the primary amine to obtain an anti-microbially active tertiary amine or quaternary ammonium group.

22. The particle of any one of claims 17 to 21, wherein said inorganic core is in a form selected from a sphere, amorphous polygonal, shallow flake-like and a rod.

23. The particle of claim 22, wherein the spherical particles have a diameter between about 5 to about 100,000 nm with pore diameter of about 1 to about 30 nm, and wherein the rod particles have a diameter of between about 10 to about 1,000 nm and length between about 10 to about 1,000,000 nm and a pore diameter of about 1 to about 30 nm.

24. A composition comprising a liquid or solid matrix embedding a plurality of particles according to any one of the preceding claims, wherein the particles are embedded in the matrix through covalent or non-covalent interactions.

25. The composition of claim 24, wherein said matrix is a polymeric matrix comprising a thermoplastic polymer selected from the group consisting of polyethylene, polypropylene, silicone, epoxy resin, composite materials and acrylic polymers such as poly methyl methacrylate.

26. The composition according to claim 24, wherein the particles are homogeneously distributed on the outer surface of the matrix at a surface concentration of between about 1 to about 100 particles per sq. μm.

27. The composition according to claim 24 having, on the average, at least one active portion per sq. μm of outer surface of matrix, the size of such active portion is at least 100 nm^2.

28. The composition of any one of claims 24 to 27, wherein the composition is a pharmaceutical composition, preferably, wherein the composition is in a form selected from the group consisting of a cream, an ointment, a paste, a dressing and a gel, more preferably, wherein the composition is formulated for topical administration.

29. A method for inhibiting or preventing biofilm formation, comprising applying onto a susceptible or infected surface or a medical device a particle according to any one of claims 1 to 23, or a pharmaceutical composition comprising such particle.

30. The method of claim 29, wherein said particle or composition is intended for administration into an oral cavity, and wherein said composition is formulated as a tooth paste, and/or applied to a surface or medical device selected from the group consisting of: a denture cleaner, post hygienic treatment dressing or gel, mucosal adhesive paste, a dental adhesive, a dental restorative composite based material for filling tooth, decay cavities, a dental restorative endodontic filling material for filling root canal space in root canal treatment, a dental restorative material used for provisional and final tooth restorations or tooth replacement, a dental inlay, a dental onlay, a crown, a partial denture, a complete denture, a dental implant and a dental implant abutment.

31. A particle according to any one of claims 1 to 23, or a pharmaceutical composition comprising such particle for use in inhibiting or preventing biofilm formation.

32. A method for inhibition of bacteria, the method comprising the step of contacting the bacteria with the particle according to any one of claims 1 to 23, or a composition comprising such particle.

33. A particle comprising

(i) an inorganic core; and

(ii) anti-microbially active groups chemically bound to the core at a surface density of at least one anti-microbially active group per 10 sq. nm,

wherein the anti-microbially active group is a quaternary ammonium group, the nitrogen atom of each quaternary ammonium group having one bond to an alkyl group having from 4 to 18 carbon atoms, and a remainder of bonds each being to an alkyl group having from 1 to 3 carbon atoms.

34. A particle according to claim 33, comprising:

(i) an inorganic core selected from silicate (SiO_4^{-4}), surface activated metal and metal oxide; and

(ii) anti-microbially active groups chemically bound to the core at a surface density of at least one anti-microbially active group per 10 sq. nm,

wherein the anti-microbially active group is a quaternary ammonium group, the nitrogen atom of each quaternary ammonium group having one bond to an alkyl group having from 4 to 18 carbon atoms, and a remainder of bonds each being to an alkyl group having from 1 to 3 carbon atoms.

35. The particle of claim 33, wherein the anti-microbially active groups is chemically bound to the core at a surface density of at least one anti-microbially active group per 1 sq. nm.

36. The particle of claim 33, wherein the nitrogen atom of each quaternary ammonium group has (i) at least one bond to the core; (ii) one bond to the alkyl group having from 4 to 18 carbon atoms, and (iii) the remainder of the bonds each being to an alkyl group having from 1 to 3 carbon atoms.

37. The particle of claim 33, wherein the alkyl group having from 4 to 18 carbon atoms is an alkyl group having 4 to 10 carbon atoms, preferably 6, 7, or 8 carbon atoms; and wherein the alkyl group having from 1 to 3 carbon atoms is preferably a methyl group.

38. The particle of claim 33, wherein the anti-microbially active group is attached to the inorganic core directly or through a linker.

39. The particle of claim 33, wherein said inorganic core comprises:

(a) silica (SiO_2) in a form selected from the group consisting of amorphous silica, dense silica, aerogel silica, porous silica, mesoporous silica and fumed silica;

(b) glasses or ceramics of silicate (SiO_4^{-4}) selected from the group consisting of aluminosilicate, borosilicate, barium silicate, barium borosilicate and strontium borosilicate;

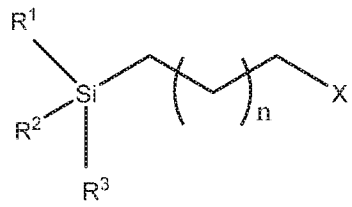
(c) surface activated metals selected from the group consisting of silver, gold, platinum, palladium, copper, zinc and iron; or

(d) metal oxides selected from the group consisting of zirconium dioxide, titanium dioxide, vanadium dioxide, zinc oxide, copper oxide and magnetite.

40. The particle of any one of claims 33 to 39, wherein said core has a solid uniform morphology with low porosity or a porous morphology having pore size diameter of between about 1 to about 30 nm.

41. The particle of claim 33, wherein the inorganic core is attached to the anti-microbially active group through a linker, wherein the linker is selected from the group consisting of a C3 to C18 alkylene substituted with at least one silane moiety; a C3 to C18 alkylene substituted with at least one phosphate moiety; a C3 to C18 alkylene substituted with at least one anhydride moiety; a C3 to C18 alkylene substituted with at least one carboxylate moiety; and a C3 to C18 alkylene substituted with at least one glycidyl moiety.

42. The particle of claim 33, wherein the linker is a C3 to C18 alkylene substituted with at least one silane moiety, and wherein a reagent represented by the structure of formula (II) is utilized to couple the inorganic core to the anti-microbially active group:



(II)

wherein

R^1, R^2 and R^3 are independently selected from the group consisting of ethoxy, methoxy, methyl, ethyl, hydrogen, sulfonate and halide, wherein at least one of R^1, R^2 and R^3 is selected from ethoxy, methoxy, sulfonate (e.g., mesyl, tosyl) and halide;

X is selected from the group consisting of NH_2, halide, sulfonate and hydroxyl; and

n is an integer between 1 and 16;

wherein said reagent is capable of being chemically bound to the surface of said inorganic core through the silicon atom, and wherein the anti- microbially active group is introduced by substituting the group X with an anti-microbially active group, or converting the group X to an anti-microbially active group.

43. The particle of any one of claims 33 to 42, wherein said inorganic core is in a form selected from a sphere, amorphous polygonal, shallow flake-like and a rod.

44. The particle of claim 43, wherein the spherical particles have a diameter between about 5 to about 100,000 nm with pore diameter of about 1 to about 30 nm, and wherein the rod particles have a diameter of between about 10 to about 1,000 nm and length between about 10 to about 1,000,000 nm and a pore diameter of about 1 to about 30 nm.

45. The particle of any one of claims 33 to 44, having a diameter between about 5 to about 100,000 nm, preferably, between about 10 to about 50,000 nm.

46. A composition comprising a liquid or solid matrix embedding a plurality of particles according to any one of claims 33 to 45, wherein the particles are embedded in the matrix through covalent or non-covalent interactions.

47. The composition of claim 46, wherein said matrix is a polymeric matrix comprising a thermoplastic polymer selected from the group consisting of polyethylene, polypropylene, silicone, epoxy resin, composite materials and acrylic polymers such as poly methyl methacrylate.

48. A composition according to claim 46, wherein the particles are homogeneously distributed on the outer surface of the matrix at a surface concentration of between about 1 to about 100 particles per sq. μm.

49. A composition according to claim 46 having, on the average, at least one active portion per sq. μm of outer surface of matrix, the size of such active portion is at least 100 nm^2.

50. The composition of any one of claims 46 to 49, wherein the composition is a pharmaceutical composition, preferably in a form selected from the group consisting of a cream, an ointment, a paste, a dressing and a gel, more preferably, wherein the composition is formulated for topical administration.

51. A method for inhibiting or preventing biofilm formation, comprising applying onto a susceptible or infected surface or a medical device a particle according to any one of claims 33 to 45, or a pharmaceutical composition comprising such particle.

52. The method of claim 51, wherein said particle or composition is intended for administration into an oral cavity, and wherein said composition is formulated as a tooth paste, and/or applied to a surface or medical device selected from the group consisting of: a denture cleaner, post hygienic treatment dressing or gel, mucosal adhesive paste, a dental adhesive, a dental restorative composite based material for filling tooth, decay cavities, a dental restorative endodontic filling material for filling root canal space in root canal treatment, a dental restorative material used for provisional and final tooth restorations or tooth replacement, a dental inlay, a dental onlay, a crown, a partial denture, a complete denture, a dental implant and a dental implant abutment.

53. A particle according to any one of claims 33 to 45, or a pharmaceutical composition comprising such particle for use in inhibiting or preventing a biofilm formation.

54. A method for inhibition of bacteria, the method comprising the step of contacting the bacteria with the particle according to any one of claims 33 to 45 or a composition comprising such particle.

55. A pharmaceutical composition formulated for topical administration comprising (i) an organic core; and (ii) anti-microbially active groups chemically bound to the core at a surface density of at least one anti-microbially active group per 10 sq. nm, wherein the anti-microbially active group is a quaternary ammonium group, the nitrogen atom of each quaternary ammonium group having one bond to an alkyl group having from 4 to 18 carbon atoms, and a remainder of bonds each being to an alkyl group having from 1 to 3 carbon atoms; wherein the composition is in a form selected from the group consisting of a cream, an ointment or a paste.

56. A particle comprising (i) an organic core; and (ii) anti-microbially active groups chemically bound to the core at a surface density of at least one anti-microbially active group per 10 sq. nm, wherein the anti-microbially active group is a quaternary ammonium group, the nitrogen atom of each quaternary ammonium group having one bond to an alkyl group having from 4 to 18 carbon atoms, and a remainder of bonds each being to an alkyl group having from 1 to 3 carbon atoms; wherein the diameter of each particle is between about >10,000 nm and about 100,000 nm.

57. A particle comprising (i) an inorganic core or an organic polymeric core; and (ii) an imine moiety chemically bound to the core, preferably at a surface density of at least one imine group per 10 sq. nm, wherein the imine group comprises a terpenoid moiety.

58. Use of a particle according to claim 57, for preparing an anti-microbially active particle according to claim 1.

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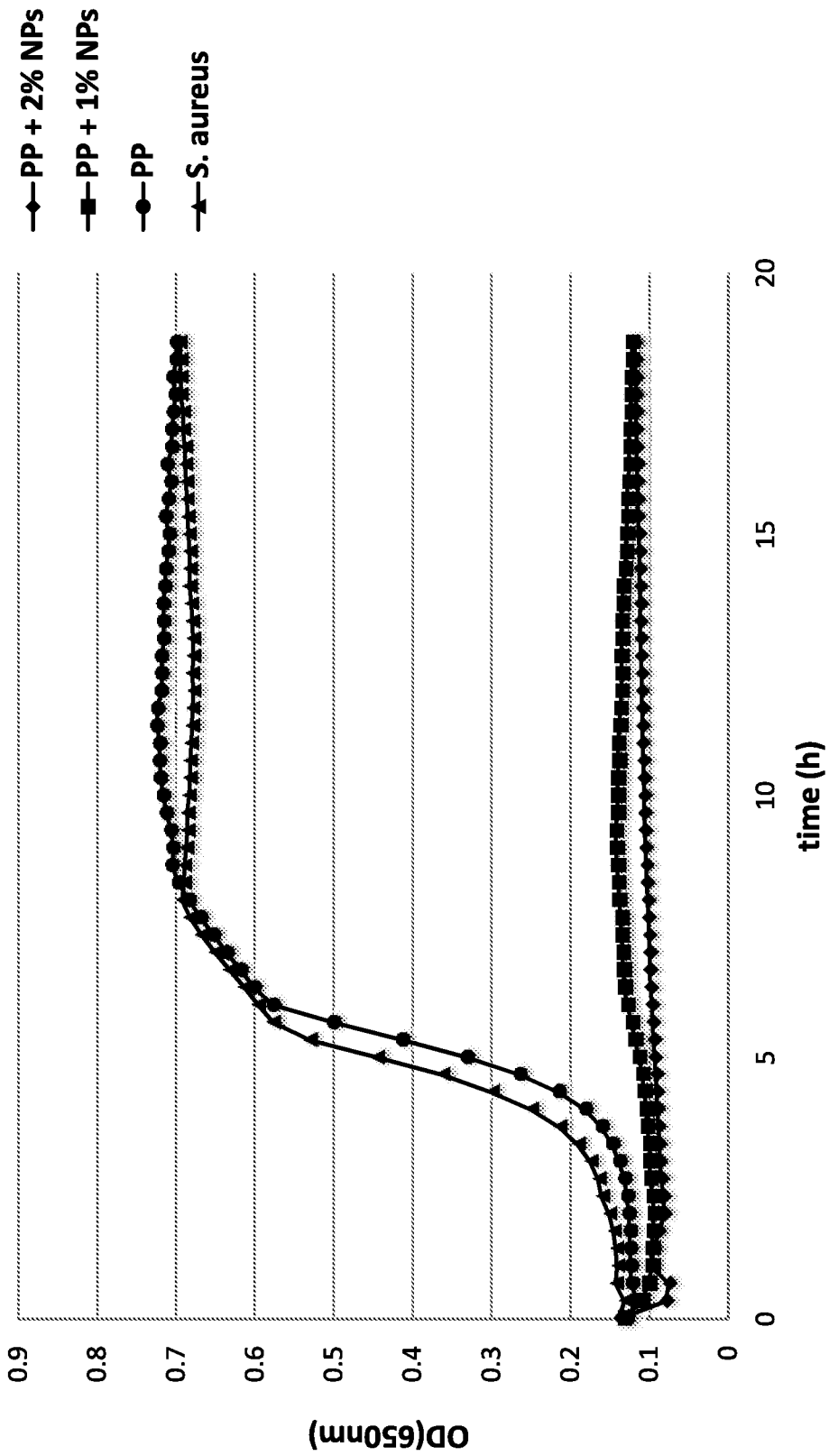
PP + 2% NPs
PP + 1% NPs
PP
S. aureus
OD(650nm)
0.9
0.8
0.7
0.6
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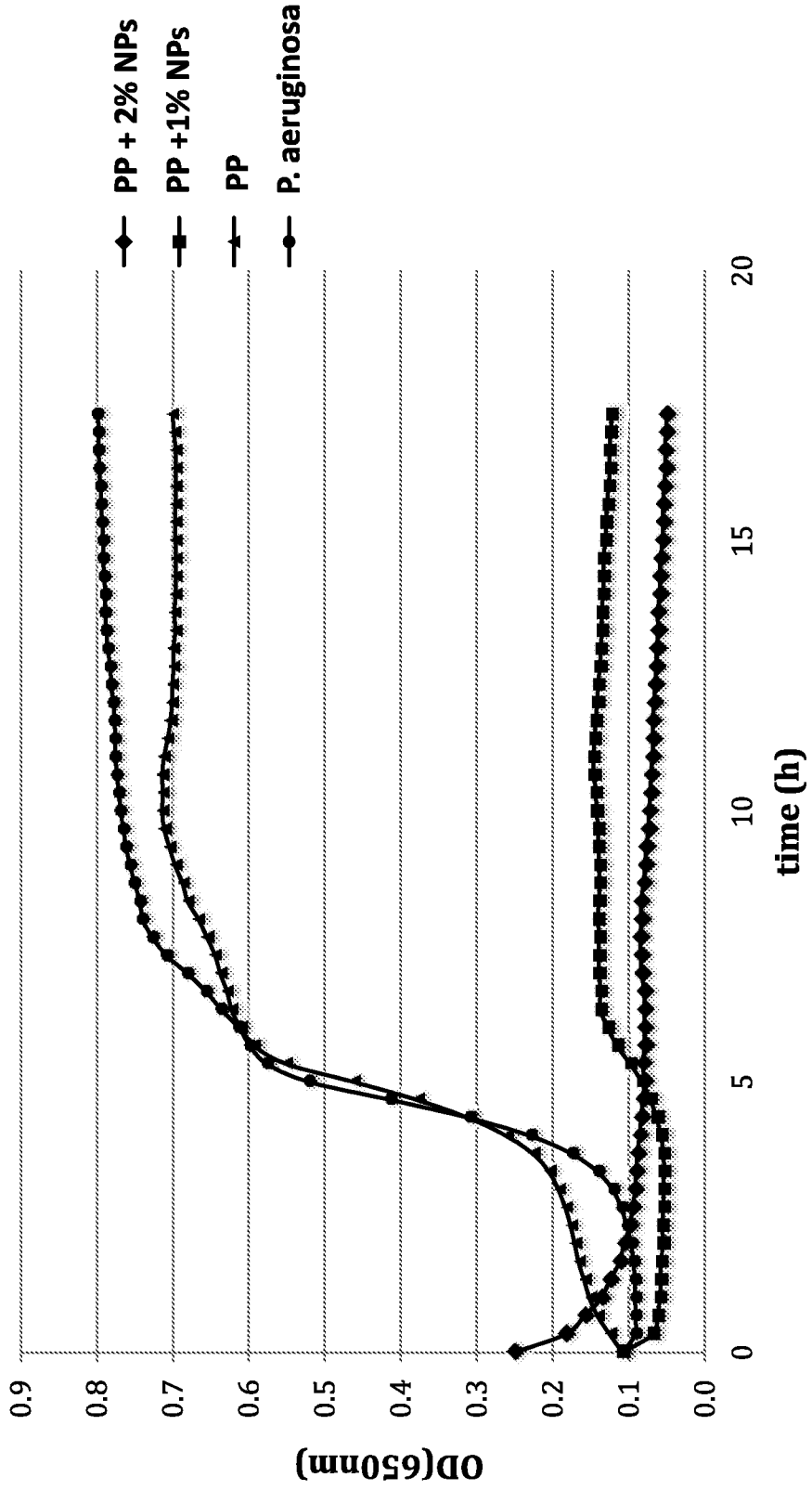
0.4
0.3
0.2
0.1
0
0
5
10
15
20
time (h)

Figure 1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PP +1% NPs
PP
P. aeruginosa
OD(650nm)
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0.1
0.2
0.3
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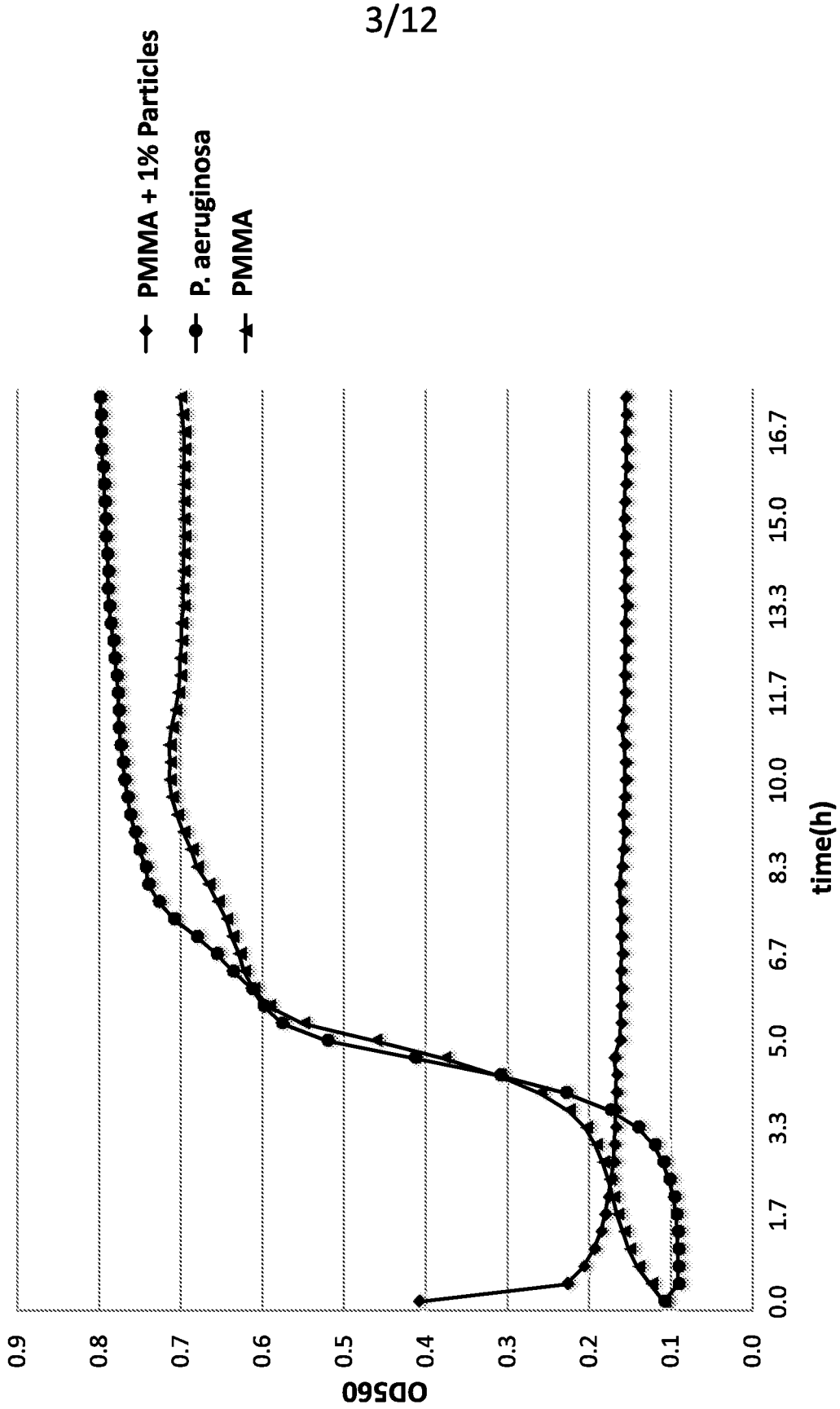
0.6
0.7
0.8
0.9
0
5
10
15
20
time (h)

Figure 2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P. aeruginosa
PMMA
OD560
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0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
time(h)
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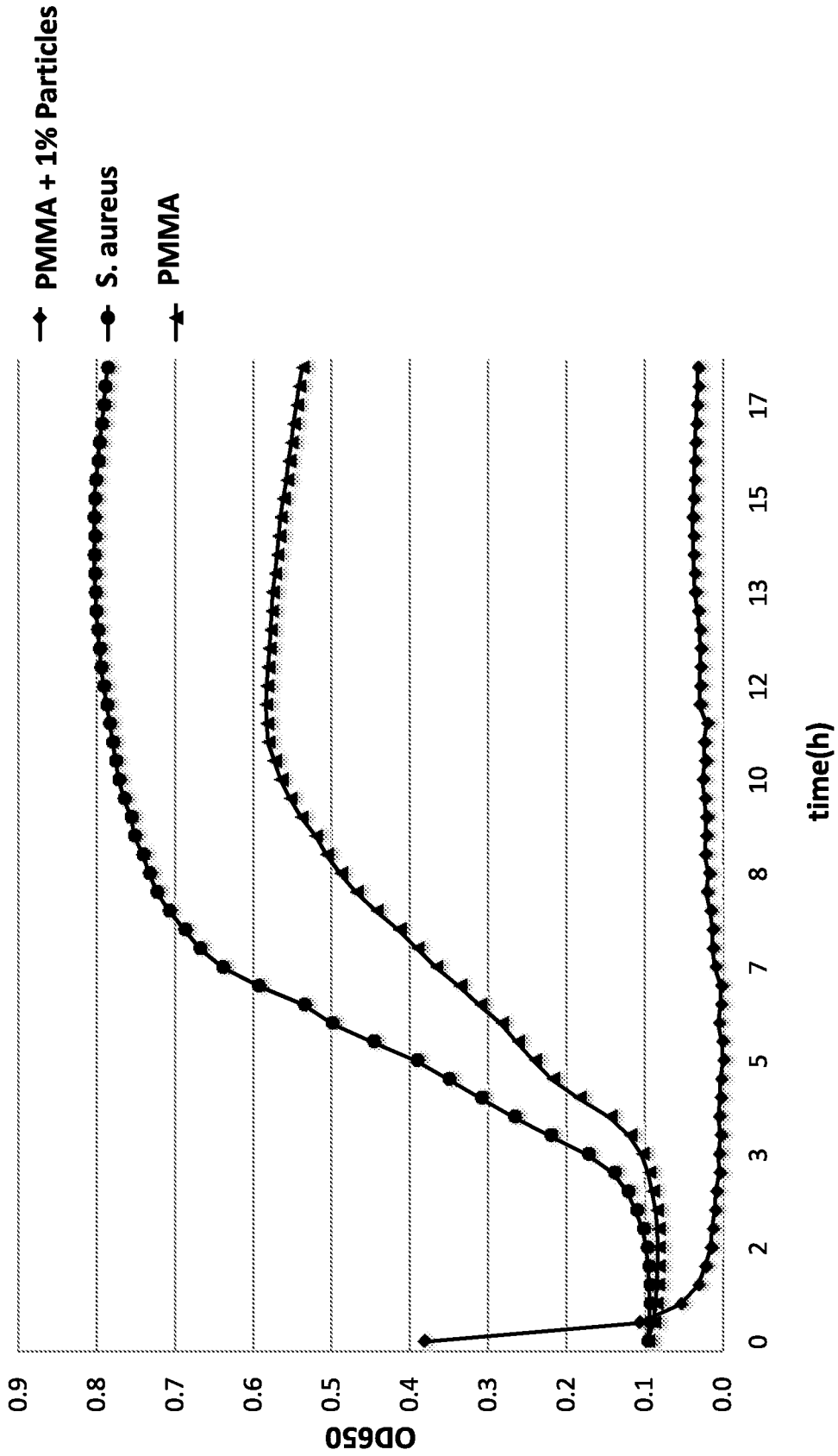
6.7
8.3
10.0
11.7
13.3
15.0
16.7

Figure 3


PMMA + 1% Particles
S. aureus
PMMA
OD650
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0.1
0.2
0.3
0.4
0.5
0.6
0.7
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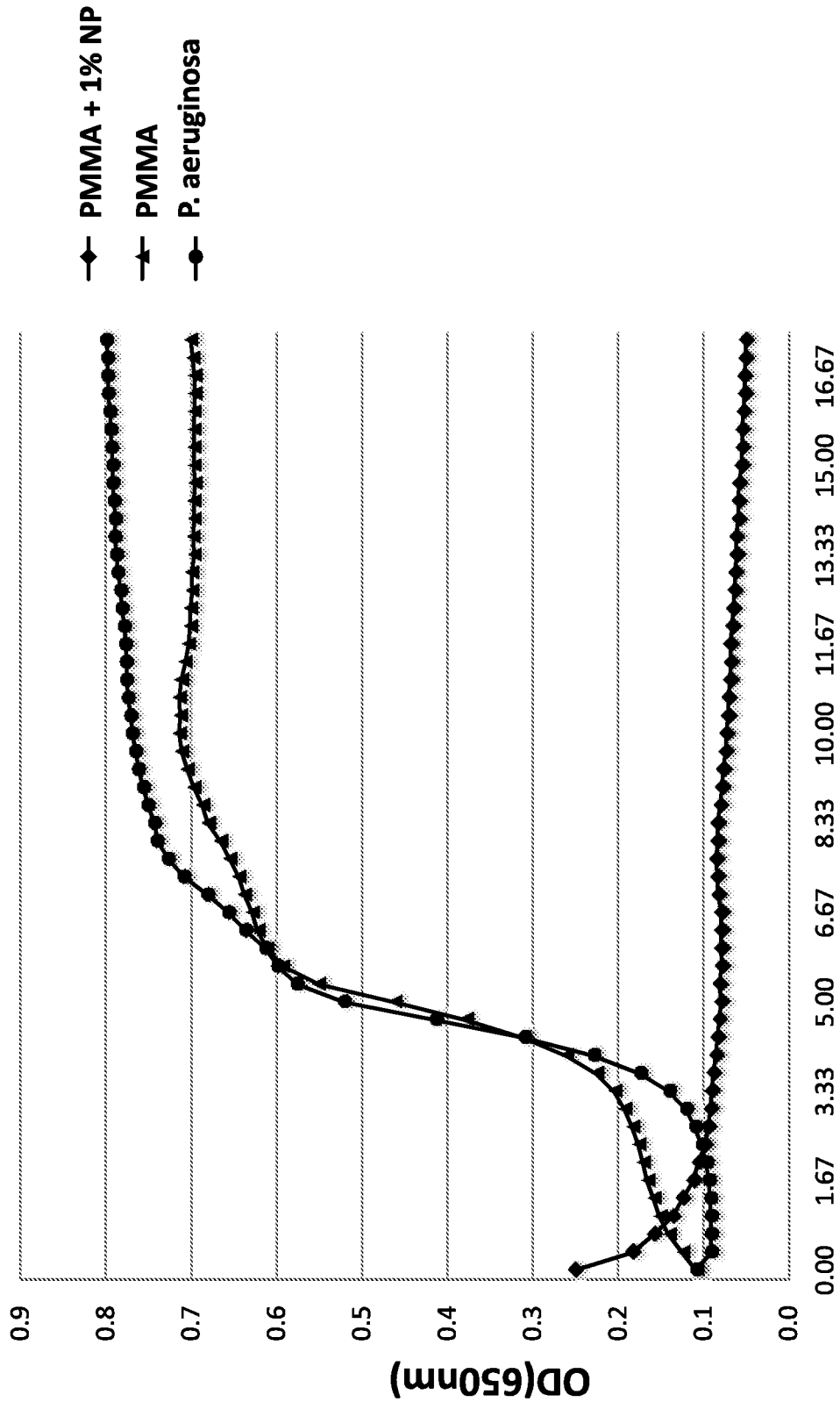
0.9
time(h)
0
2
3
5
7
8
10
12
13
15
17

Figure 4


PMMA + 1% NP
PMMA
P. aeruginosa
OD(650nm)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
0.00
1.67
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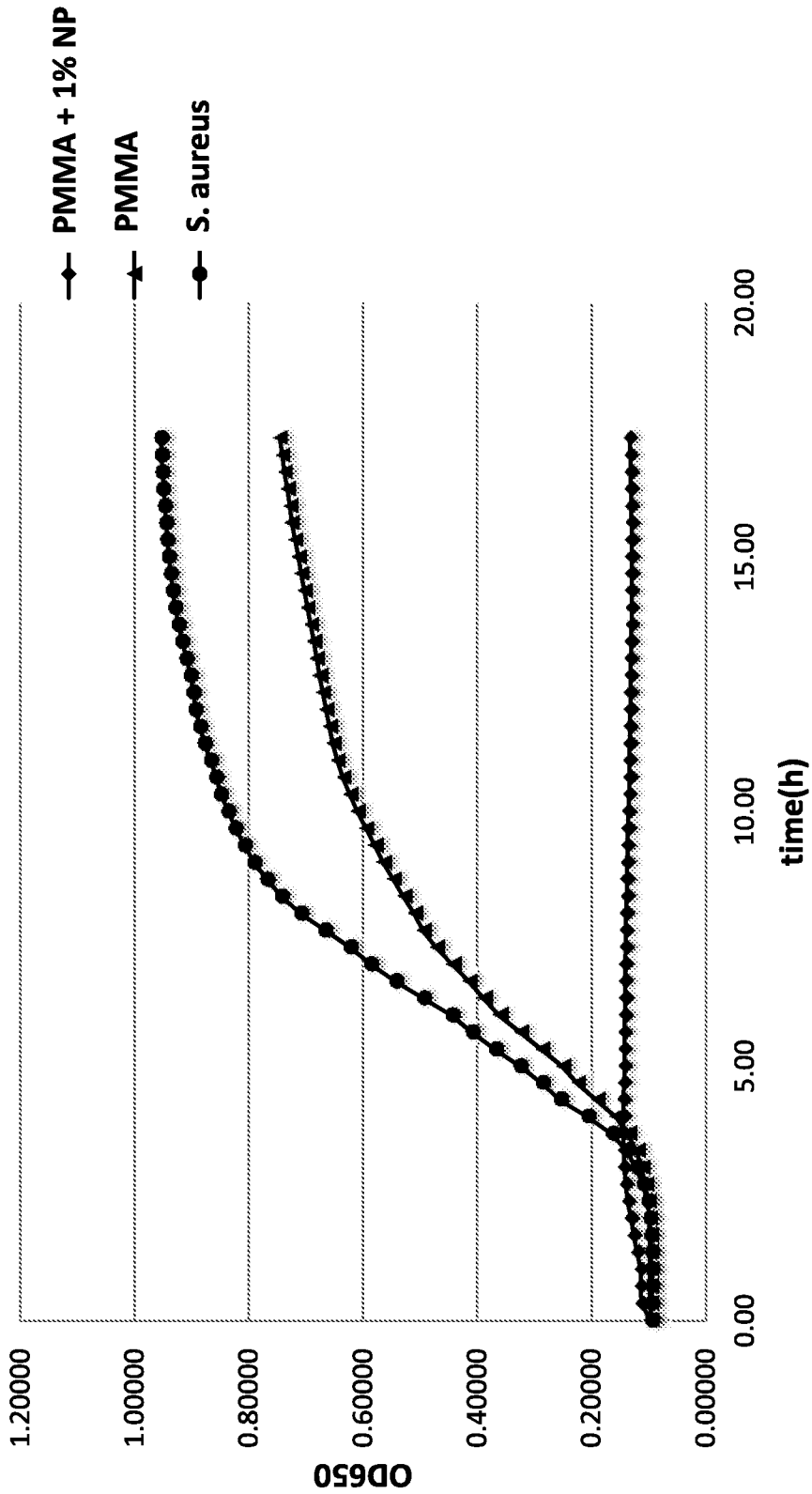
11.67
13.33
15.00
16.67
Time (h)

Figure 5


PMMA + 1% NP
PMMA
S. aureus
OD650
1.20000
1.00000
0.80000
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0.20000
0.00000
0.00
5.00
10.00
15.00
20.00
time(h)

Figure 6

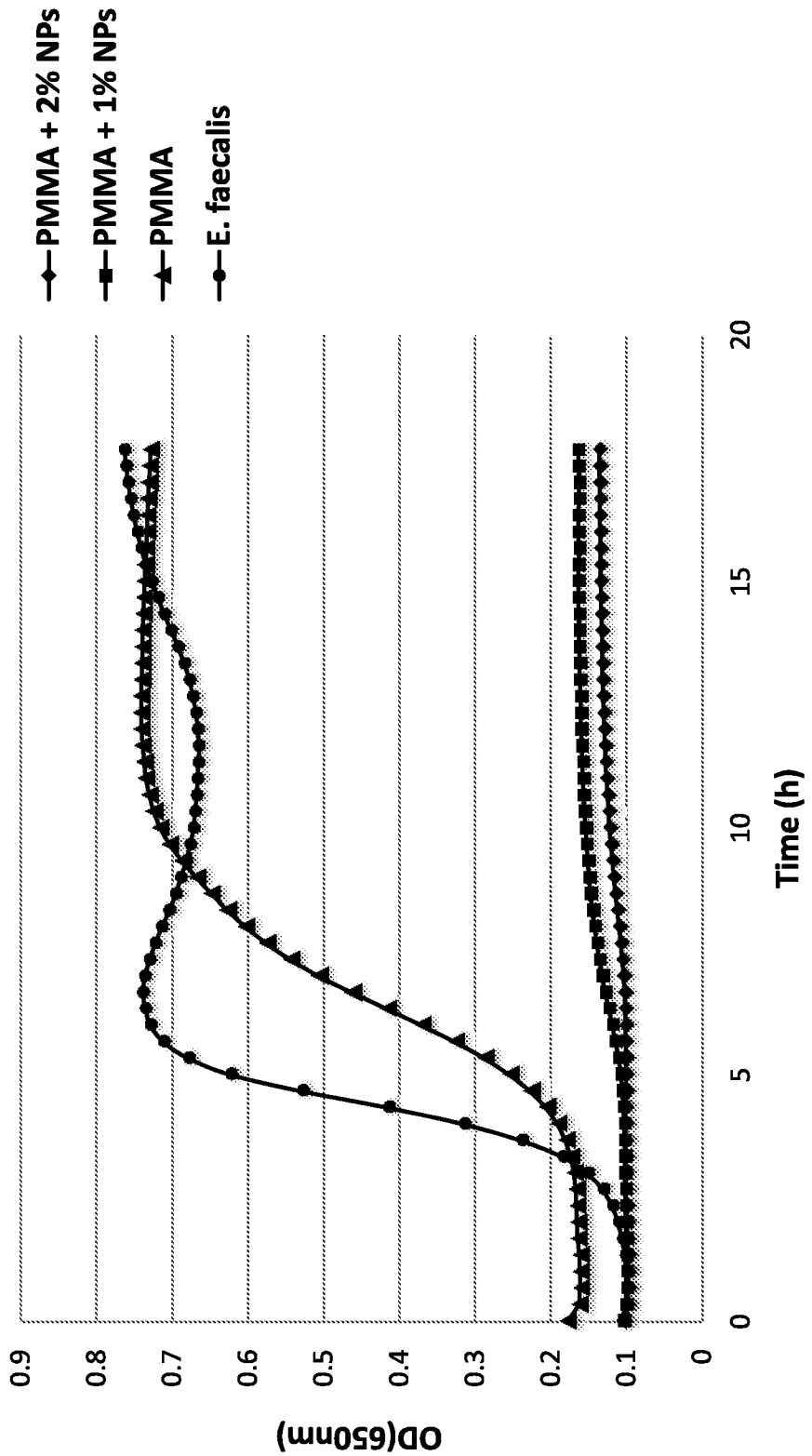
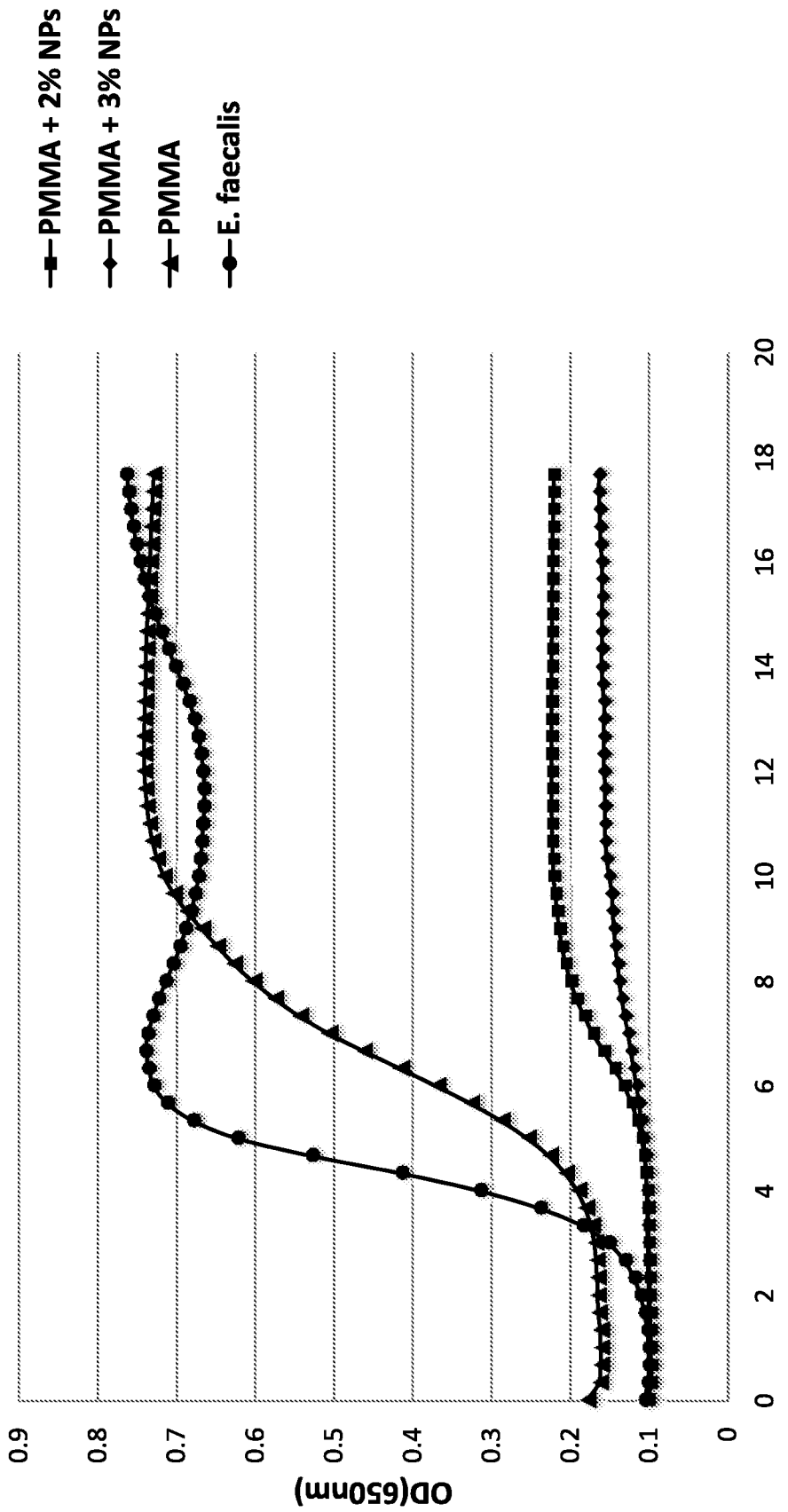

PMMA + 2% NPs
PMMA + 1% NPs
PMMA
E. faecalis
OD(650nm)
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
5
10
15
20
Time (h)

Figure 7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PMMA + 3% NPs
PMMA
E. faecalis
OD(650nm)
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0.8
0.7
0.6
0.5
0.4
0.3
0.2
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0
0
2
4
6
8
10
12
14
16
18
20
time (h)

Figure 8

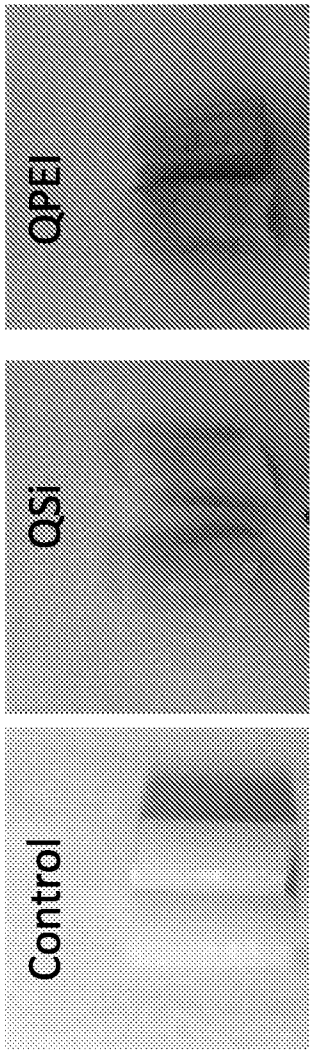

Figure 9A

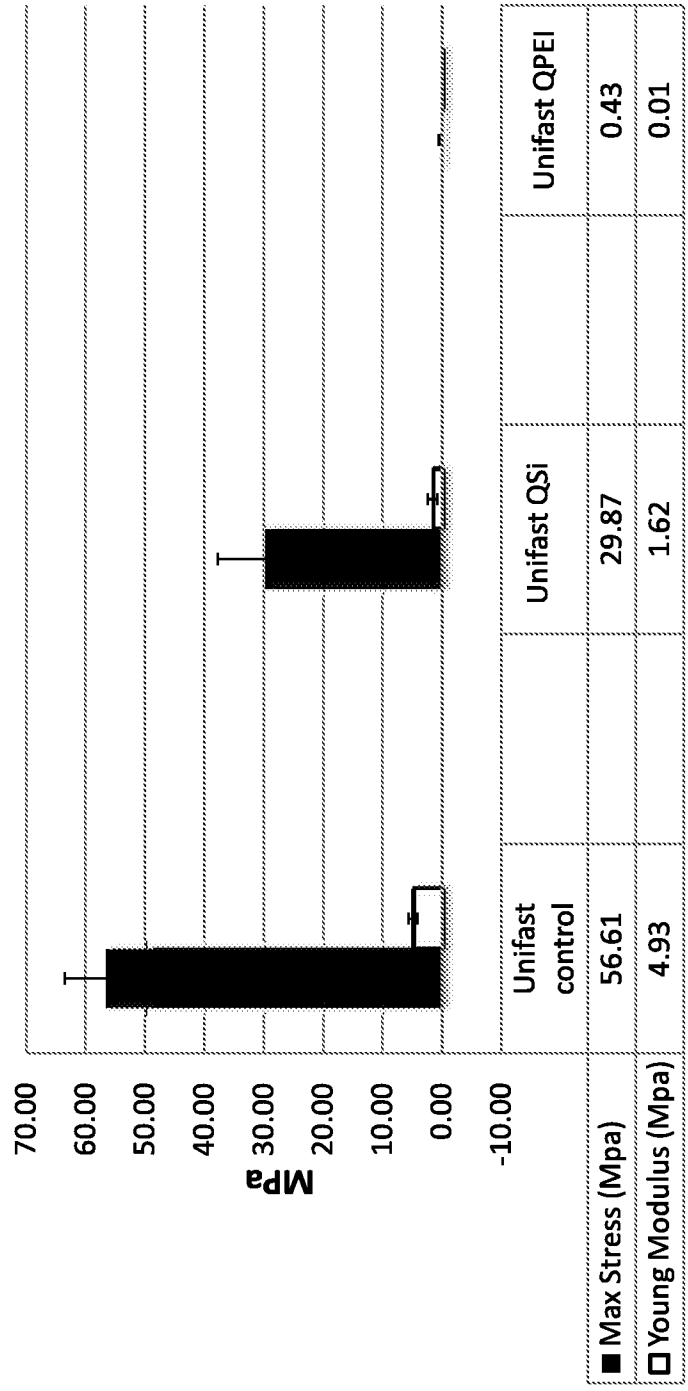

Figure 9B

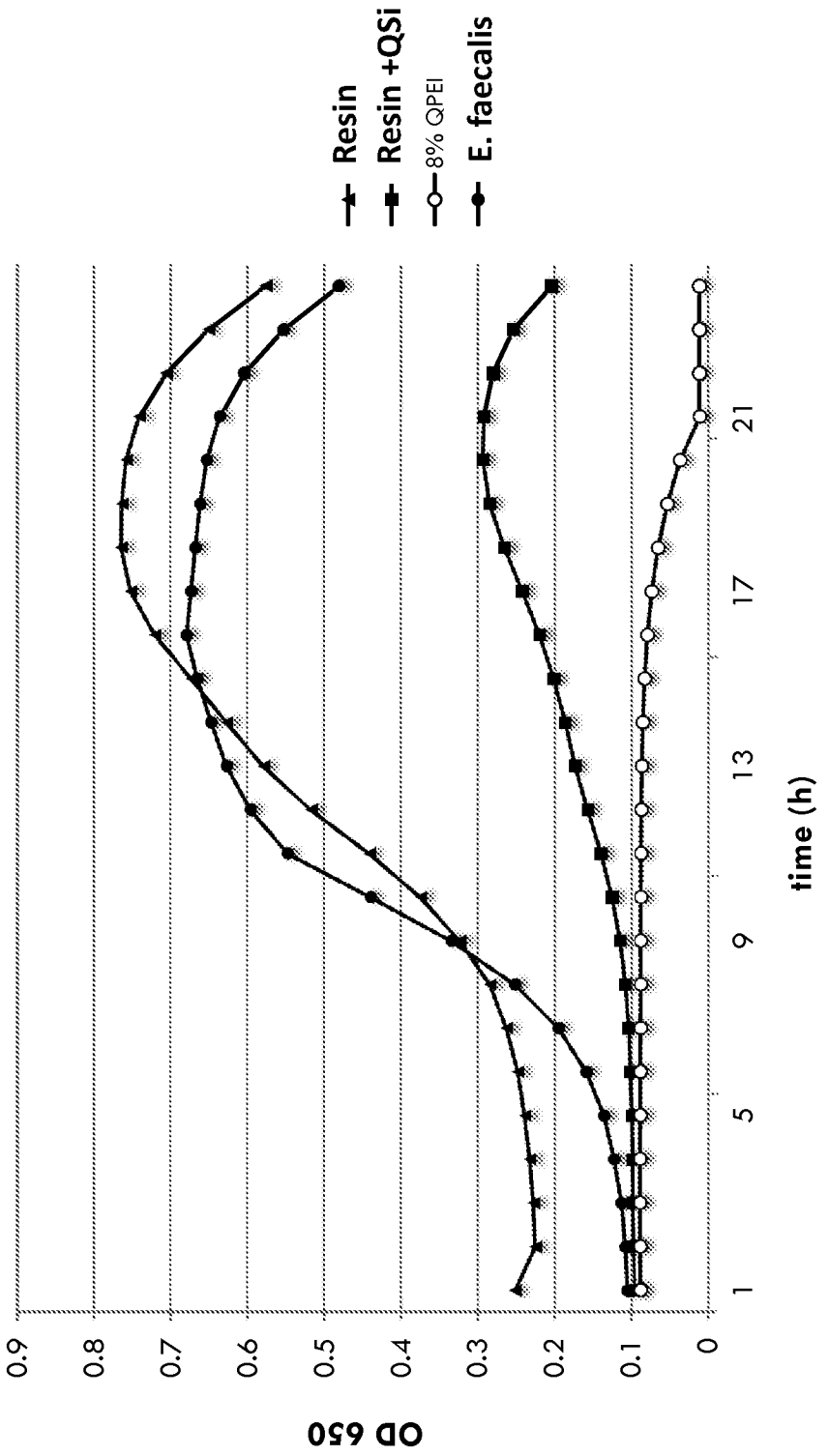
Resin
Resin +QSi
8% QPEI
E. faecalis
OD 650
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1
5
9
13
17
21
time (h)

Figure 10A

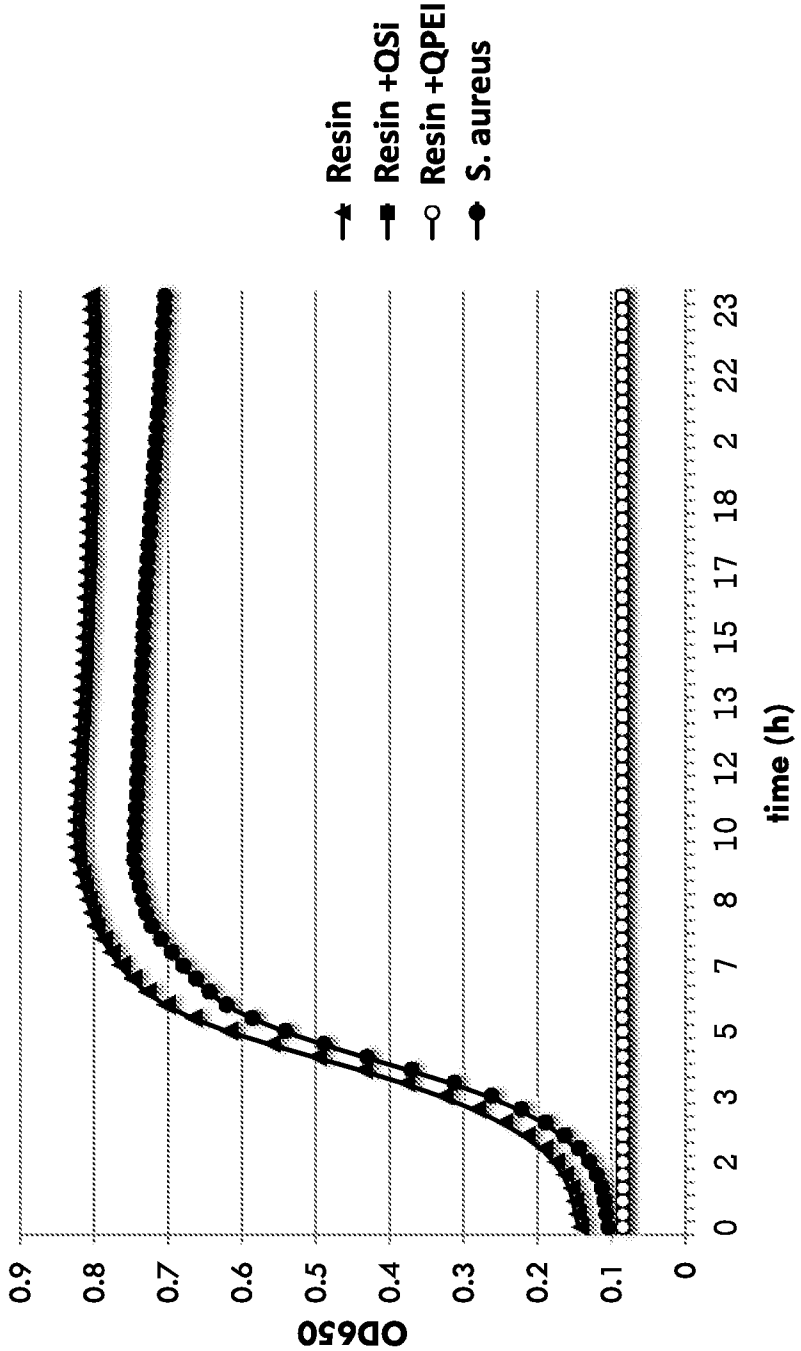
Resin
Resin +QSi
Resin +QPEI
S. aureus
OD650
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0 2 3 5 7 8 10 12 13 15 17 18 2 22 23
time (h)

Figure 10B

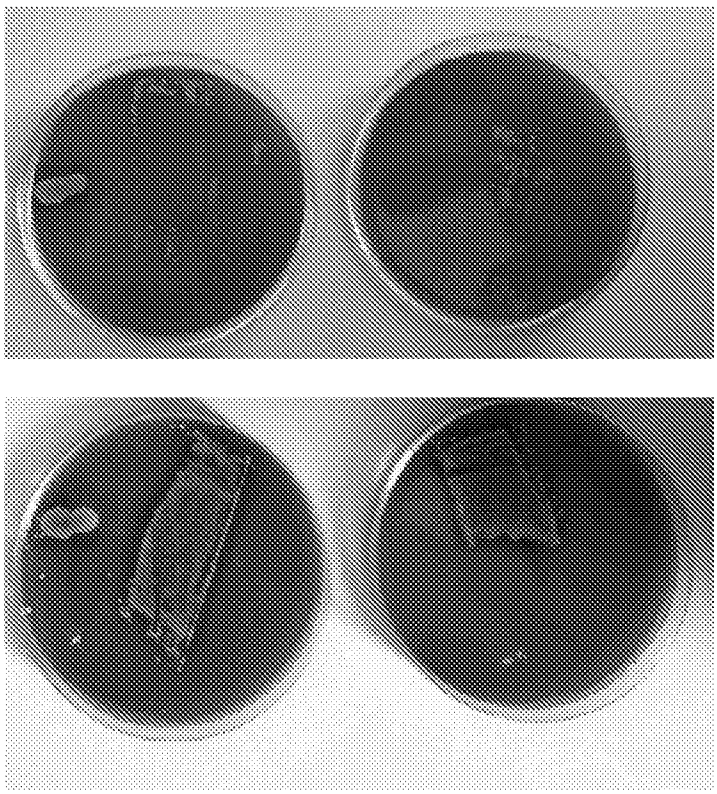

Figure 11

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IL2016/050219

A. CLASSIFICATION OF SUBJECT MATTER

IPC (2016.01) C11D 1/62, A61P 31/04, C11D 1/40, A01N 25/12, B82B 1/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC (2016.01) C11D 1/62, A61P 31/04, C11D 1/40, A01N 25/12, B82B 1/00

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Databases consulted: THOMSON INNOVATION, Google Scholar
Search terms used: Antimicrobial activity, antimicrobial agent, dental products, functionalized dental devices, quaternary ammonium group, terpenoid, inorganic core, silicate.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP 1841314 B1 Domb, Abraham J. [IL]; Weiss, Ervin I. [IL]; Beyth, Nurit [IL]; Farber, Ira [IL]; Perez Davidi, Michael [IL] 19 Mar 2014 (2014/03/19) Paragraphs [0011]-[0012], [0015], [0017]-[0018], claim 29	1-58
Y	WO 9510940 A1 Jacobson, Howard, Wayne [US]; Scholla, Michael, Heal [US]; Wigfall, Annie, Williams [US] 27 Apr 1995 (1995/04/27) Pg. 3 lines 22-26 and claim 1.	1-58
Y	US 2007258996 A1 Pradip, Mookerjee [US]; April Zambelli-Weiner [US]; Shira Kramer [US] 08 Nov 2007 (2007/11/08) paragraph [0006]	1-58

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 Jun 2016	16 Jun 2016

Name and mailing address of the ISA:	Authorized officer
Israel Patent Office Technology Park, Bldg.5, Malcha, Jerusalem, 9695101, Israel Facsimile No. 972-2-5651616	Furmanovich Elinor Telephone No. 972-2-5651752

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/IL2016/050219

Patent document cited search report	Publication date	Patent family member(s)	Publication Date
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		EP 1841314 B1	19 Mar 2014
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		AU 2005320933 B2	28 Jul 2011
		CA 2594216 A1	06 Jul 2006
		CN 101160048 A	09 Apr 2008
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		IL 184195 A	30 Apr 2015
		JP 2008527068 A	24 Jul 2008
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		US 8535645 B2	17 Sep 2013
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		AU 4278593 A	15 Aug 1994
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		AU 7054494 A	08 May 1995
		BR 9406056 A	26 Dec 1995
		CA 2159808 A1	13 Oct 1994
		CA 2159808 C	28 Mar 2006
		CA 2171703 A1	27 Apr 1995
		CN 1095083 A	16 Nov 1994
		CN 1039025 C	08 Jul 1998
		DE 69321139 D1	22 Oct 1998
		DE 69321139 T2	12 May 1999
		DE 69404642 D1	04 Sep 1997
		DE 69404642 T2	05 Mar 1998
		EP 0677989 A1	25 Oct 1995

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/IL2016/050219

Patent document cited search report	Publication date	Patent family member(s)	Publication Date
		EP 0677989 B1	16 Sep 1998
		EP 0693096 A1	24 Jan 1996
		EP 0693096 B1	30 Jul 1997
		EP 0724388 A1	07 Aug 1996
		ES 2105687 T3	16 Oct 1997
		JP H08508537 A	10 Sep 1996
		JP 3507900 B2	15 Mar 2004
		JP H08505858 A	25 Jun 1996
		JP H09504022 A	22 Apr 1997
		TW 275075 B	01 May 1996
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		US 5595750 A	21 Jan 1997
		US 5643592 A	01 Jul 1997
		WO 9415462 A1	21 Jul 1994
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		WO 9422962 A1	13 Oct 1994
US 2007258996 A1	08 Nov 2007	US 2007258996 A1	08 Nov 2007
		AU 2007342391 A1	17 Jul 2008
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		EP 2124575 A4	09 Jan 2013
		MX 2009006992 A	20 Jan 2010
		US 2008194518 A1	14 Aug 2008
		US 2009312279 A1	17 Dec 2009
		US 2012121723 A1	17 May 2012
		WO 2008085446 A2	17 Jul 2008
		WO 2008085446 A3	28 Aug 2008