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(54) Title: 3D-PRINTABLE ANTIMICROBIAL COMPOSITE RESINS, METHODS FOR MANUFACTURING THE SAME

(57) Abstract: The invention relates to the fields of microbiology, dentistry and the manufacture of antibacterial products, more in particular to printable polymerizable composite resins that have antimicrobial properties. Provided is a photocurable antibacterial resin composition, comprising (i) a polymer matrix comprising at least one biocompatible polymerizable acrylic compound; (ii) a photoinitiator system; and (iii) a methacrylate-modified positively charged compound (PCC) having antibacterial properties, wherein the PCC is present in the composition as polymeric PCC (p PCC) obtained by polymerization of methacrylate-modified PCC monomers.



Title: 3D-printable Antimicrobial composite resins, methods for manufacturing the same.

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The invention relates to the fields of microbiology, dentistry and the manufacture of antibacterial products. More in particular, it relates to printable polymerizable composite resins that have antimicrobial properties.

10 Over the past several years, additive manufacturing techniques, more commonly referred to as “3D printing” (3DP),¹ have entered the realm of public awareness by rapidly penetrating a variety of application areas beyond small-scale manufacturing and prototyping. 3D printing (or additive manufacturing, AM) is any of various processes used to make a three-
15 dimensional object. In 3D printing, additive processes are used, in which successive layers of material are laid down under computer control. These objects can be of almost any shape or geometry, and are produced from a 3D model or other electronic data source. 3DP enables the low-cost, bottom-up fabrication of objects with complex geometries that are difficult to produce
20 by traditional fabrication methods. Advancements in 3DP technology have also had an impact on drug discovery²⁻⁴, medical devices^{5,6}, tissue engineering⁷⁻¹¹, dental restorations^{12,13}, microfluidics and customized reactionware for chemical synthesis and analysis^{14,15}. US2014/131908 discloses printable polymerizable material systems for making dental
25 products such as artificial teeth, dentures, splints, veneers, inlays, onlays, copings, frame patterns, crowns and bridges and the like.

Although 3D printed models composed of metals, ceramics, polymers¹⁶, and even cell-loaded hydrogels¹⁷⁻¹⁹ have been realized, the development of materials with integrated functions amenable for 3DP has
30 been slow.

The surface properties of 3D printed materials are especially vital to their implementation in the medical field. Specifically, engineering an intrinsic antimicrobial functionality into implantable medical devices can reduce the risk of bacterial infections²⁰, which pose major health threats and are the leading cause of implant failure. It has been estimated that at least 50% of all nosocomial infections are implant-related and affect around two million patients each year in the United States alone²¹. Similarly, the field of dentistry is severely affected by biofilm formation. Up to 15% of oral biofilm-related post-treatment complications in orthodontic patients require professional care with annual costs of over 500 million dollars in the USA²². Dental patients in the USA spend over 20 billion dollars annually to replace failed resin composite restorations that were damaged by bacterial infiltration and the resulting secondary caries²³. Therefore, microbe-induced infection has promoted the ever-growing need for development of antimicrobial materials in the large area of biomedical engineering.

Previously, numerous efforts have been undertaken to equip conventional dental restorations with antimicrobial properties. These focused on release of various antibacterial agents such as fluorides²⁴, zinc ions^{25,26}, silver ions²⁷, chlorhexidine²⁸, and antibacterial peptides²⁹. However, the release of antibacterial agents may impair the mechanical properties of the restorations or may exert toxicity on the surrounding tissue if the dose is not properly controlled.

Recognizing the significant negative consequences of bacterial biofilms and the highly individualized nature of custom intraoral appliances and prostheses calling for an all-digital workflow, the present inventors set out to develop a polymer design strategy used to develop biocompatible antimicrobial resins that are suitable for 3D printing, and can thus be used

for manufacturing intraoral appliances and dental restorations. It was specifically aimed to provide a polymerizable antimicrobial resin showing minimal leakage of antimicrobial agents and that is compatible with stereolithography processing (SLP), which represents a widely employed
5 3DP technology. In the context of SLP, rapid solidification of photopolymer liquid is an essential prerequisite for successful printing³⁶.

Surprisingly, it was found that at least some of the above goals could be met by the provision of novel 3D-printable antimicrobial resins that were
10 obtained by mixing photo-polymerizable positively-charged monomers or polymers into composite resins. More specifically, monomers of a methacrylate-based positively charged compound (PCC) having antibacterial properties are first converted into a high molecular weight polymer (pPCCs) and then pPCCs were mixed with the frame components
15 for photo-curing. The thus formed semi-interpenetrating polymer network (SIPN) exhibits nearly “zero” leaching properties and all pPCC-containing SIPN exhibited contact-killing activities towards model bacterial strains. Importantly, successful 3D printing (e.g. by SLP) of pPCCs-containing monomer mixtures could be realized.

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After photo-curing, positively-charged groups are non-covalently incorporated into the material which exhibited good antimicrobial activities when in contact with the bacteria. Biocompatibility issues were curtailed by limiting the leaching of positively charged polymers out of the composites
25 while the mechanical properties of the pristine polymer matrix were maintained, qualifying these materials for future clinical use. Moreover, the resins were found to be SLP compatible.

Accordingly, the invention provides a photocurable antibacterial
30 composition, comprising (i) a polymer matrix comprising at least one

biocompatible polymerizable acrylic compound; (ii) a photoinitiation system and (iii) a methacrylate-based positively charged compound (PCC) having antibacterial properties, wherein the methacrylate-based PCC is present in the composition as polymeric PCC (pPCC) obtained by polymerization of
5 methacrylate-modified PCC monomers.

Photocurable antibacterial compositions comprising a methacrylate-based positively charged compound having antibacterial properties are known in the art.

10 WO2012/177960 relates to composition and method of making a polymerizable antibacterial/ antimicrobial resin and using such a bioactive resin in formulated dental compositions. Disclosed are compositions comprising acrylic monomers, a photoinitiator and methacrylate-based monoimidazole resins. Nothing is mentioned about converting antibacterial
15 monomers into a high molecular weight polymer, and mixing them with the frame components to form a semi-interpenetrating polymer network (SIPN).

US2011/256510 discloses dental compositions comprising acrylic monomers, including TEGDMA and UDMA, a photoinitiator and quaternary ammonium resins.

20 US2005/095266 discloses a surface coating that results from photocopolymerisation of a monomer with a biocidal group, including quaternary ammonium methacrylates, a photoprimer and a copolymerizable compound, like an acrylate.

WO2015/061097 similarly discloses coating compositions, wherein
25 the PCC is a quaternary ammonium compound or an imidazolium which are copolymerized with PEGMA in the presence of a photoinitiator.

JP2007/302651 discloses an antimicrobial photosetting composition comprising a methacrylate-based imidazolium monomer which is copolymerized with other acrylate monomers in the presence of a
30 photoinitiator.

US2013/195793 discloses an antimicrobial photocurable coating composition from the copolymerization of a urethane (meth)acrylate with a quaternary ammonium group, a hydroxyl-modified acrylate and a photoinitiator.

- 5 However, the prior art is completely silent about trapping a PCC in the polymer matrix in the form of a prepolymerized polymer, and the advantageous effects associated therewith on the biological safety of a 3D printed material prepared therefrom.
- 10 In a resin of the invention, the presence of positively charged compounds (PCCs) with an appended alkyl chain is responsible for the antibacterial property through the mechanism of killing on contact against a variety of bacterial strains. Although the exact killing mechanism of PCCs grafted on a surface is still unclear, it is generally accepted that grafted PCCs interact
- 15 with the bacterial cell wall and disrupt the cytoplasmic membrane to release potassium and other constituents, which causes cell death albeit through a different mechanism than PCCs in solution³³⁻³⁵.

As used herein, the term “photocurable” refers to a polymerizable material that can be polymerized at a rapid rate by exposure to light due to the

20 presence of a photoinitiation system. Photoinitiation systems are known per se in the art, and typically contain two or three components. In one embodiment, it comprises at least one photosensitizer, and optionally a co-initiator. Exemplary photosensitizers include camphorquinone, phenylpropanedione, monoacrylphosphine oxide (TPO), bisacrylphosphine

25 oxide (Ir819), 2,4,6-trimethylbenzoyldiphenylphosphine oxide, or methyl benzoin which causes polymerization to be initiated upon exposure to activating wavelengths of light. In one embodiment, bisacrylphosphine oxide photoinitiator (Ir819) is used. The initiating components may be present in an amount of at least 0.05% by weight, and preferably at least about 0.3%

30 by weight of the overall polymerizable composition. The overall

polymerizable composition may include less than about 20% and preferably less than about 5% by wt of the photoinitiation system. For example, the initiating components may be present in a range of about 0.05% to about 10%, and preferably from about 0.3% to about 5% by wt of the overall
5 polymerizable composition.

In a specific aspect, the composition comprises a camphorquinone (CQ) compound and an aromatic amine co-initiator, such as ethyl- 4-(dimethylamino)benzoate (EDMAB), which is a well known two-component
10 initiation system for use in dental photocurable matrix resins. For example, CQ and EDMAB are used in a amount of about 0.25-1.5mol%, preferably about 0.5-1mol%, in relation to the total amount of double bonds in the monomer mixture.

15 A photocurable antibacterial composition of the invention comprises a polymer matrix comprising at least one biocompatible polymerizable acrylic compound. Preferred polymerizable acrylic compounds are selected from the group consisting of mono-, di- or poly-acrylates and methacrylates. Examples include methyl acrylate, methyl methacrylate, methacrylic acid,
20 ethyl acrylate, ethyl methacrylate, isopropyl methacrylate, tert-butyl (meth)acrylate, glycerol dimethacrylate (GDMA), cyclohexyl (meth)acrylate, 4-tert-butylcyclohexyl (meth)acrylate, tetrahydrofurfuryl (meth)acrylate, n-hexyl acrylate, 2-phenoxyethyl (meth)acrylate, stearyl acrylate, allyl acrylate, isobornyl (meth)acrylate, stearyl (meth)acrylate, phenoxy benzyl
25 (meth)acrylate, o-phenylphenol ethyl (meth)acrylate. Also encompassed are tris (2-hydroxy ethyl) isocyanuratediacrylate, the reaction product of octadecyl isocyanate and caprolactone 2-(methacryloyloxy)ethyl ester, the reaction product of octadecyl isocyanate and 2-hydroxyethyl acrylate; the reaction product of octadecyl isocyanate and hydroxypropyl (meth)acrylate;
30 the reaction product of octadecyl isocyanate and 2-hydroxypropyl 2-

(methacryloyloxy)-ethyl phthalate; the reaction product of octadecyl isocyanate and 2-hydroxy-3-phenoxypropyl acrylate; the reaction product of octadecyl isocyanate and glycerol dimethacrylate; the reaction product of octadecyl isocyanate and pentaerythritoltriacylate; the reaction product of
 5 cyclohexyl isocyanate and 2-hydroxyethyl (meth)acrylate; the reaction product of benzyl isocyanate and 2-hydroxyethyl (meth)acrylate; 1,14-tetradecanedimethacrylate, dimethyloltricyclodecanediacylate, glycerol diacylate, glycerol triacylate, ethylene glycol diacylate, diethyleneglycoldiacylate, triethyleneglycoldimethacrylate, tetraethylene
 10 glycol di(meth)acrylate, 1,3-propanediol diacylate, 1,3-propanediol dimethacrylate, trimethylolpropane tri(meth)acrylate, 1,2,4-butanetriol trimethacrylate, 1,4-cyclohexanediol diacylate, 1,4-cyclohexanediol dimethacrylate, 1,6-hexanediol di(meth)acrylate, pentaerythritoltriacylate, pentaerythritoltetraacylate, pentaerythritoltetramethacrylate, sorbitol
 15 hexacrylate, 2,2-bis[4-(2-hydroxy-3-acryloyloxypropoxy)phenyl]propane; 2,2-bis[4-(2-hydroxy-3-methacryloyloxypropoxy)phenyl]propane (Bis-GMA); the reaction product of Bis-GMA and octadecyl isocyanate; the reaction product of Bis-GMA and cyclohexyl isocyanate; 2,2-bis[4-(acryloyloxy-ethoxy)phenyl]propane; 2,2-bis[4-(methacryloyloxy-ethoxy)phenyl]propane
 20 (or ethoxylatedbisphenol A-dimethacrylate) (EBPADMA).

Preferred polymerizable acrylic compounds for use in the present invention include urethane di(meth)acrylate (UDMA), diurethanedimethacrylate (DUDMA), polyurethane dimethacrylate (PUDMA); and mixtures thereof. Very good results can be obtained using a
 25 monomer mixture comprising DUDMA and GDMA. In one embodiment, the polymer matrix comprises DUDMA and GDMA, preferably at least 40wt% DUDMA and/or 20-40wt% GDMA. A formulation comprising 20-40wt% GDMA was found to have sufficiently low viscosity so that they can be handled and cured and the final device can be removed easily from the
 30 liquid resin bath (reservoir). At the same time, the formulations are capable

of producing dental products having sufficient mechanical strength and integrity. Accordingly, in one embodiment the invention provides a photocurable antibacterial resin composition, comprising

- (i) a polymer matrix comprising diurethanedimethacrylate (DUDMA) and glycerol dimethacrylate (GDMA), preferably at least 40wt% DUDMA and/or 20-40wt% GDMA; (ii) a photoinitiator system; and (iii) a methacrylate-based positively charged compound (PCC) having antibacterial properties. The PCC may be present in the DUDMA/GDMA matrix as covalently attached monomer obtained by in situ co-polymerization with the matrix resin components or as polymeric PCC (pPCC) obtained by polymerization of methacrylate-modified PCC monomers.

Further preferred polymer matrices for use in the present invention are those comprising UDMA, preferably in combination with one or more of TEGDMA (triethyleneglycol dimethacrylate), E4-A (tetraethylene glycol diacrylate), or UDMA/TTA (trimethylolpropanetriacrylate). For example, good results are obtained using UDMA/TEGDMA, UDMA/E4-A, or UDMA/TTA monomer mixtures.

The composition of the invention comprises one or more methacrylate-based PCCs as antimicrobial additive. In one embodiment, the methacrylate-based PCC is modified with 2-hydroxy methacrylate (HEMA). The amount of methacrylate-based or methacrylate-modified PCCs in a composition can vary e.g. depending on the type of PCC and/or desired antimicrobial strength. In one embodiment, it is present in said composition in an amount of 5 to 25 mol%, preferably 10 to 20 mol%.

As described in more detail herein below, three series of exemplary methacrylate-based PCCs were synthesized with different alkyl chain lengths ($n = 4, 8, 12, 16$) based on quaternary ammonium (QA- C_n), pyridinium (Py- C_n), and imidazolium (Im- C_n). Rapid conversion rates of the

methacrylate groups is observed, which confirms rapid polymerization (< 10 sec to reach higher than 55% conversion) for all formulations (data not shown) and meets the needs of rapid curing required for SLP.

Accordingly, in a specific aspect the methacrylate-based PCC is a

- 5 methacrylate-based quaternary ammonium, pyridinium or imidazolium. Preferably, the modified PCC is a methacrylate-modified imidazolium. The methacrylate-based quaternary ammonium, pyridinium or imidazolium can be alkylated with an alkyl chain comprising at least 4 carbon atoms, preferably at least 6, more preferably at least 8 carbon atoms.

- 10 Tensile tests indicated that the 3D-printed materials using a resin of the invention have similar mechanical properties compared to samples that were fabricated in a polymerization mold by conventional photo-illumination, which reflected that CAD-sliced layers were fused together well during the layer-by-layer photo-curing. Further, contact killing
15 properties investigated by Petrifilm method showed that very few colonies were found on the UDMA/GDMA/QA_C12 (14 mol%) disc, compared to the control disc only.

- To optimize the biological safety of the 3D-printed material, PCC monomers are first converted into a high molecular weight polymer (pPCCs)
20 and then pPCCs were mixed with the frame components for photo-curing. The thus formed semi-interpenetrating polymer network (SIPN) exhibits nearly “zero” leaching properties and all pPCC-containing SIPN exhibited contact-killing activities towards model bacterial strain. Similarly, the 3D printability of pPCCs-containing resins was investigated and successful
25 printing could be realized.

- In a preferred embodiment, the methacrylate-based PCC is present in the composition as a high molecular weight polymer having an average molecular weight M_w above 2500 g/mol, obtained by polymerization of methacrylate-modified PCC monomers, such that the positively-charged
30 groups are included non-covalently into the photo-curable composite resins

by trapping the positively-charged group-containing polymers into the semi-interpenetrating polymer network. In a preferred aspect, the cationic polymer is designed in such a way that it interacts with the polymer network via hydrogen bonding. In one embodiment, pPCC is synthesized by
5 copolymerization of cationic monomers and 2-hydroxyethyl methacrylate (HEMA). However, the skilled person will understand that various other combinations of cationic monomers and polymerizable monomers can be used to obtain hydrogen bonding interactions between PCC-polymers and the SIPN matrix.

10

To even further reduce liberation of cationic moieties out of the resin, a new polymerization technique for providing pPCC was developed. Since free radical polymerization of *p*(HEMA-*co*-QAC₁₂MA) resulted in a broad molecular weight distribution containing a significant amount of low
15 molecular weight components, we fabricated the cationic polymer by a process called reversible addition fragmentation transfer (RAFT) polymerization³⁸. The RAFT technique yields narrowly dispersed polymers with high molecular weights, and a polymer characteristic that was also achieved for *p*(HEMA-*co*-QAC₁₂MA) (PDI = 1.16, M_w = 9800 g/mol). It was
20 found that the cationic polymer produced by the RAFT process leached out from the resin to a lesser extent compared to the cationic macromolecule fabricated by the free radical polymerization process (See Figure 4). Surprisingly, only 400 ppm of a model compound (fluorescent dye) was detected after four weeks in the leaching tests. Without wishing to be bound
25 by theory, this can be explained by either the decreased diffusion of the high molecular weight pPCC produced by RAFT polymerization or by covalent incorporation of the pPCC chain into the photo-cured resin. Accordingly, in a preferred embodiment, a resin composition of the invention comprises polymeric PCC (pPCC) obtainable or obtained by reversible addition
30 fragmentation transfer (RAFT) polymerization of methacrylate-modified

PCC monomers. The invention also relates to a polymeric PCC (pPCC) obtainable by polymerization of methacrylate-modified PCC monomers, preferably wherein said polymerization comprises reversible addition fragmentation transfer (RAFT) polymerization.

5

The composition may contain further additives, for example one or more of pigments, inorganic fillers and/or organic fillers, catalysts, stabilizers, plasticizers, fibers or their combinations. Preferred stabilizers are butylated hydroxytoluene (BHT) and the methyl ether of hydroquinone (MEHQ). It
10 may also include compounds to introduce radiopaque in the material.

The filler component may be present in an amount of at least 0% by weight, and more preferably at least about 2% by wt of the overall polymerizable composition. Furthermore, the filler component may be present in an
15 amount less than about 75% by weight and more preferably less than about 65% by wt of the overall polymerizable composition. For example, the filler component may be present in a range of about 0 to about 75, and preferably from about 2 to about 65% by wt of the overall polymerizable composition.

20 Conventional filler materials such as inorganic fillers, which can be naturally-occurring or synthetic, can be added to the printable polymerizable composition. Exemplary filler materials include silica, titanium dioxide, iron oxides, silicon nitrides, glasses such as calcium, lead, lithium, cerium, tin, zirconium, strontium, barium, and aluminum-based
25 glasses, borosilicate glasses, strontium borosilicate, barium silicate, lithium silicate, lithium alumina silicate, kaolin, quartz, and talc. Preferably, the silica is in the form of silanized fumed silica. Preferred glass fillers are silanized barium boron aluminosilicate and silanized fluoride barium boron aluminosilicate. Preferably, these inorganic fillers can be suspended in
30 printable polymerizable resin. Organic particles such as poly(methyl

- methacrylate) (PMMA), highly crosslinked PMMA beads, poly(methyl/ethyl methacrylate), poly(methyl/butyl methacrylate), rubber modified PMMAs, rubber impact modifiers, crosslinked polyacrylates, thermoplastic and crosslinked polyurethanes, grounded polymerized compounds of this
- 5 invention, polyethylene, polypropylene, polycarbonates and polyepoxides, and the like also can be used as fillers. These organic fillers can be added into printable polymerizable resin described above. Preferably, these organic fillers can dissolve or suspend in printable polymerizable resin.
- 10 The pigment can be used as coloring or shading agents. Clear (transparent) compositions do not need any pigments. The pigment component may be present in an amount of at least 0% by weight, and more preferably at least about 0.001% by wt the overall polymerizable composition. The overall polymerizable composition also may include less than about 5% by weight
- 15 and more preferably less than about 1% by wt of the pigment component. For example, the pigment component may be present in a range of about 0 to about 5%, and preferably from about 0.001 to about 1% by wt of the overall polymerizable composition.
- 20 The invention also relates to a method for providing an antibacterial polymer, comprising providing a composition according to the invention and inducing photocuring. Photocuring (photopolymerization) can be initiated by irradiating the composition with light of the appropriate wavelength, which will typically depend on the photoinitiator system used.
- 25 In one embodiment, the composition can be cured by exposure to blue, visible light in the wavelength region of about 400 to about 500 nm. For example, a composition comprising a photoinitiator system involving a camphorquinone compound generates free radicals when exposed to a wavelength in this range.

In a method of the invention, photocuring may but does not have to involve 3D printing. For example, also encompassed are methods for providing an antibacterial polymer, wherein a liquid polymer composition is brought into a mold or matrix and then exposed to light to induce curing and

5 solidification of the composition. The antibacterial polymer can also have the shape of a sheet or a film.

Also provided herein is an antibacterial polymer obtainable by a method according to the invention. Such polymer is characterized by comprising, or consisting of, a cured composition according to the invention, which by
10 virtue of the cationic PCCs is a contact-killing, non-leaching antimicrobial material, capable of killing microorganisms which come into contact with the material. The polymer can be in any desired shape or object. For example, it can be in the form of an antimicrobial coating, film, 3D printed dental object, and the like. However, as will be appreciated by the skilled
15 person, the principles outlined herein can be transferred in the future to any other application areas where antimicrobial properties are desired, such as consumer goods and packaging, education, electronics, hearing aids, sporting goods, jewelry, medical, toys.

20 In one embodiment, the polymer comprises PCC that is covalently linked to the polymer matrix. In another embodiment, the PCC is present as a polymer that is trapped inside the crosslinked polymer matrix (SIPN).

In a preferred embodiment, photocuring is performed at least in part during a 3D printing process. In general, two approaches (DLP printer or
25 Stereolithography printer (SLP)) can be used for fabricating the three-dimensional object using the materials of this invention.

Following each of these approaches, the printable polymerizable material is flowable or heated to form a flowable liquid. The printer builds successive

layers of the polymerizable material by projecting or irradiating light onto the building plane and cures to form the antibacterial polymer.

In one aspect, a method of the invention comprises SLP. In the course of SLP, a z-stage is moved in a liquid polymer resin tank and layer-by-layer

5 photo-curing provides one with a 3D object. Due to the outstanding geometry adaptability, different dental restorations can be easily fabricated in a single process just by changing the computer-aided design (CAD) drawing file.

10 Thus, also provided herein is a method for providing a 3D antimicrobial object, comprising the steps of

a. loading a photocurable antibacterial resin composition of the invention as a liquid into a resin bath of a 3D printer based on stereolithography or other light irradiations;

15 b. using laser beam or light irradiation tracing out the shape of each layer of the liquid resin composition to form a polymerized solid; and

c. applying one or more successive layers of the polymerized material until an object of predetermined shape is formed.

20 The method may further comprise the step of fully curing the partially cured resultant predetermined polymerized shape. Additionally, wash solvents (e.g., ethyl acetate, alcohols, acetone, THF, heptane, etc. or their combinations) may be used to remove uncured resin from 3D (dental) objects and final cure or heat treatment may be used to enhance their mechanical
25 and physical properties as well as their performance. Air barrier coating or sealer may be used prior to final cure. Inert atmosphere may be used for final cure dental devices or mass production of devices (e.g., denture teeth, denture bases, crowns) in a manufacturing environment.

Also provided herein is a 3D printed object obtainable by a method of the invention and/or comprising a cured antimicrobial polymer composition of the invention. In one embodiment, the 3D printed object is a dental product, preferably selected from the group consisting of dental prosthesis, artificial
5 teeth, dentures, splints, veneers, inlays, onlays, copings, frame patterns, crowns and bridges.

LEGEND TO THE FIGURES

10

Figure 1. PCC-incorporating polymer system. a) Structures of three series of positively charged methacrylate monomers; b) incorporation of PCC monomers into the matrix resins; c) XPS detection and decomposition of surface N 1s signal; d-f) CFUs by contact-killing of three formulations of
15 PCCs-incorporated resins against *S. mutans* NS.

Figure 2. A) 3D-printed molar tooth model (top) and a clear dental splint (bottom); B) Tensile properties of 14 mol% QA_C₁₂-containing polymer network fabricated by 3D-printing and a normal curing process; C) CLSM
20 images of *S. mutans* NS biofilms on the surface of 3D-printed control resins without cationic groups (left) and resins containing 14 mol% of QA_C₁₂ (right). The scale bar represents 50 μ m. Samples were inoculated with 1×10^8 /mL of *S. mutans* in adhesion buffer for 5 h, followed by incubation in growth medium for 6 days at 37°C (the medium was exchanged with fresh
25 medium every other day). Before CLSM, the biofilm was stained with SYTO 9/propidium iodide dye mixtures, resulting in green and red colored bacteria indicating live and dead bacteria, respectively.

Figure 3. a) chemical structure of three series of PCC-containing polymer; b)
30 preparation of PCC polymer-incorporated SINP system.

Figure 4, panels a, b and c) CFUs of *S. mutans* NS in the contact-killing tests of SIPN with three series of PCC polymer immobilized; d) 3D printed dental appliances or models (from left to right: molar tooth; clear splint; crown; tensile test bar; contact-killing test disc) based on PCC polymer-incorporated SINP.

Figure 5. a) chemical structures of methacrylate-modified rhodamine B and b) RhB-labeled polymer; c) leaching tests of RhB-directly incorporated polymer network (■), normal RhB-polymer incorporated SIPN (▼) and RAFT RhB-polymer incorporated SIPN (▲). Here, the “normal RhB-polymer” represents RhB-labeled polymer synthesized by normal thermal-induced free-radical polymerization in presence of azobisisobutyronitrile (AIBN). RhB-MA monomer and RhB-polymer have the same excitation/emission wavelengths : λ_{ex} =561 nm; λ_{em} =583 nm.

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Figure 6. Pictures of Petrifilm contact-killing experiments of 3D-printed composite resins without (panel a) or with (panel b) QA_C12 (14 mol%). The dots represent *S. mutans* CFUs after incubation at 37°C for 48 h (30 bacteria per cm² in feed). To prove that the killing effect is not induced by the leaching of the QA_C12 (14 mol%)-containing resins, the QA_C12 resins were immersed in PBS buffer for 2 days and *S. mutans* was then incubated with the washing solution on the Petrifilm for 2 days (panel c).

Figure 7. Tensile determined mechanical properties of SIPN containing pPCC (25wt%) fabricated by 3D-printing or normal curing. Panel A: breaking stress. Panel B: Breaking elongation.

25

EXPERIMENTAL SECTION

1. Materials and characterizations

1.1 Materials

5 Diurethanedimethacrylate (DUDMA, mixture of isomers, $\geq 97\%$),
camphorquinone (CQ, 97%), ethyl 4-(dimethylamino)benzoate (EDMAB, $\geq$
99%), glycerol dimethacrylate (GDMA, mixture of isomers, 85%), 2-
(dimethylamino)ethyl methacrylate (DMAEMA, 98%), 4-pyridinemethanol
(99%), 1-(2-hydroxyethyl)imidazole (97%), glycerol dimethacrylate (mixture
10 of isomers), 2-hydroxyethyl methacrylate (97%), methacrylic acid (98%),
N,N'-dicyclohexylcarbodiimide (99%), 4-(dimethylamino)pyridine ($>99\%$), 4-
cyano-4-(dodecylthiocarbonothioylthio)pentanoic acid (CDTA, 97%), 1-
bromooctane (99%), 1-bromodecane (98%), 1-bromododecane (97%) and 1-
bromohexadecane (97%) were purchased from Aldrich. 1-bromobutane (99%)
15 and hydroquinone (ReagentPlus®, $\geq 99.5\%$) were obtained from Sigma-
Aldrich. All other chemicals including solvents were used as received.

1.2 Characterizations

^1H NMR measurements were conducted on a Bruker 400 MHz spectrometer
20 at 25°C and CDCl_3 , *d*₄-methanol, or *d*₆-DMSO were used as solvents for
dissolving different samples. Chemical shifts were given in parts per million
from that of tetramethylsilane (TMS) as an internal reference. Fourier
transform infrared (FT-IR) measurement were conducted on a Bruker IFS88
instrument.

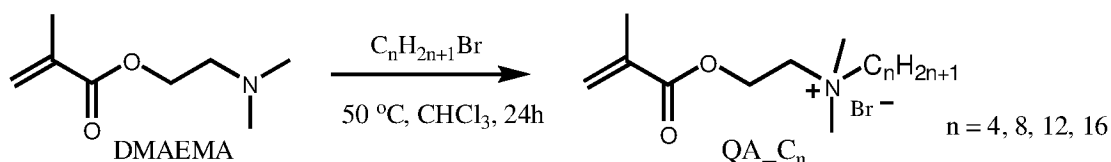
25 Gel permeation chromatography (GPC) measurements were
performed in dimethylformamide with 0.01 M LiBr on a Viscotek GPCmax
equipped with model 302 TDA detectors, using 2 columns (Pl-gel 5 μ 30 cm
mixed-C from Polymer Laboratories) with flow rate 1 mL/min. The

molecular weight calibrations were carried out using poly(methyl methacrylate) (PMMA) standards in a molecular weight range of 850 to 1.9×10^6 g/mol.

The quaternary nitrogen on the surface was determined by X-ray photoelectron spectroscopy (XPS). The instrument (S-probe; Surface Science Instruments, Mountain View, CA) was equipped with a monochromatic X-ray source (Al K anode yielding 1486.8 eV X-rays), and was operated at 10 kV accelerating voltage and 22 mA filament current. The direction of the photoelectron collection angle was set to 35 degrees with respect to the sample surface, and the electron flood gun was set at 10 eV. A survey scan was made with a 1000×250 m² spot and a pass energy of 150 eV. The peaks were integrated after nonlinear background subtraction to yield elemental surface compositions, expressed in atom%.

Example 1. Synthesis of polymerizable positively-charged compounds (PCCs)

1.1 Quaternary ammonium methacrylate (QA_{C_n})



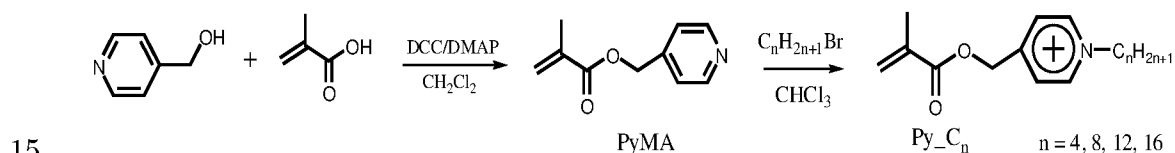
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QA_{C_n} with alkyl chain lengths in the range of C₄ to C₁₆ were synthesized according to a previously published procedure [1] with slight modifications. As an example, the synthesis of QAMA_{C₄} is described here: 10 g of DMEAMA (63.6 mmol) and 9.58 g of 1-bromobutane (67 mmol) were dissolved in 100 mL chloroform. To inhibit self-polymerization of DMEAMA during quaternization, a small amount of hydroquinone (70 mg, 0.636 mmol)

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was added to the mixture. The reaction was conducted at 50°C for 24 h, and then the solvent was evaporated under reduced pressure, followed by three times precipitation in a large excess of n-hexane. The precipitates were collected, re-dissolved in 20 mL of chloroform, and then passed through a layer of alkaline aluminum oxide to get rid of hydroquinone. The final product was obtained by removal of the solvent to give a white powder (yield: 89%). ¹H NMR (400 MHz, CDCl₃): δ (ppm) = 0.91 (t, 3H, N⁺CH₂CH₂CH₂CH₃), 1.40 (m, 2H, N⁺CH₂CH₂CH₂CH₃), 1.75 (m, 2H, N⁺CH₂CH₂CH₂CH₃), 1.93 (s, 3H, CH₂C(CH₃)COO), 3.49 (s, 6H, CH₂N⁺(CH₃)₂C₆H₁₃), 3.68 (t, 2H, N⁺CH₂CH₂(CH₂)₃CH₃), 4.15 (t, 2H, CH₂CH₂N⁺(CH₃)₂C₆H₁₃), 4.65 (t, 2H, CH₂CH₂N⁺(CH₃)₂C₆H₁₃), 5.66 (s, 1H, CHHC(CH₃)COO), 6.12 (s, 1H, CHHC(CH₃)COO).

1.2 Pyridinium methacrylate (Py_C_n)



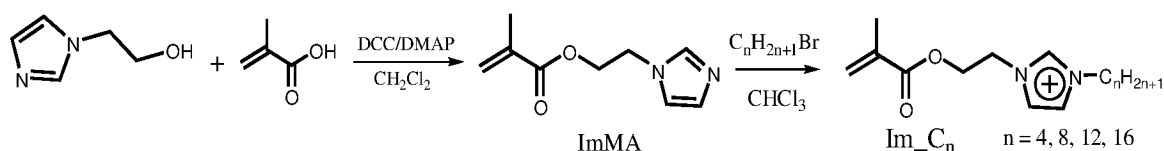
Py_C_n with alkyl chain lengths in the range of C₄ to C₁₆ were synthesized in two steps:

First, DCC/DMAP mediated esterification of 4-pyridinemethanol (Py-OH) and methacrylic acid (MAA) was carried out to give the intermediate compound pyridin methacrylate (PyMA): 5.0 g of Py-OH (28.2 mmol), 2.43 g of MAA (28.2 mmol) and 0.34 g of 4-(dimethylamino)pyridine (2.82 mmol) were dissolved in 100 mL of anhydrous CH₂Cl₂, followed by dropwise addition of DCC/CH₂Cl₂ solution (7.0 g in 50 mL CH₂Cl₂) at 0°C over 1 h. After a large amount of insoluble precipitate was formed, the reaction was allowed to proceed at room temperature for another 12 h. The insoluble precipitate was removed by filtration and the filtrate was concentrated by evaporation. Flash column chromatography on silica gel was performed to purify the product with EtOAc/n-hexane (1/3, v/v) as the mobile phase. ¹H

NMR (400 MHz, d_6 -DMSO): δ (ppm) = 1.92 (s, 3H, $\text{CH}_2\text{C}(\text{CH}_3)\text{COO}$), 5.22 (s, 2H, $\text{C}(\text{CH}_3)\text{COOCH}_2$), 5.74 and 6.13 (s, 2H, $\text{CH}_2\text{C}(\text{CH}_3)\text{COO}$), 7.36 (d, 2H, $\text{COOCH}_2\text{C}(\text{CH})_2(\text{CH})_2\text{N}$), 8.55 (d, 2H, $\text{COOCH}_2\text{C}(\text{CH})_2(\text{CH})_2\text{N}$).

Second, quaternarizations of PyMA were carried out with alkyl bromides of different alkyl chain lengths to obtain Py- C_n . The reaction conditions and purification were similar to the synthesis of QA- C_n . ^1H NMR (400 MHz, d_6 -DMSO): δ (ppm) = 0.83 (t, 3H, $\text{N}^+\text{CH}_2\text{CH}_2(\text{CH}_2)_n\text{CH}_3$), 1.24 (m, 2nH, $\text{N}^+\text{CH}_2\text{CH}_2(\text{CH}_2)_n\text{CH}_3$), 1.88 (m, 2H, $\text{N}^+\text{CH}_2\text{CH}_2(\text{CH}_2)_n\text{CH}_3$), 1.95 (s, 3H, $\text{CH}_2\text{C}(\text{CH}_3)\text{COO}$), 4.57 (t, 2H, $\text{N}^+\text{CH}_2\text{CH}_2(\text{CH}_2)_n\text{CH}_3$), 5.49 (s, 2H, $\text{C}(\text{CH}_3)\text{COOCH}_2$), 5.83 and 6.22 (s, 2H, $\text{CH}_2\text{C}(\text{CH}_3)\text{COO}$), 8.10 (d, 2H, $\text{COOCH}_2\text{C}(\text{CH})_2(\text{CH})_2\text{N}^+$), 9.06 (d, 2H, $\text{COOCH}_2\text{C}(\text{CH})_2(\text{CH})_2\text{N}^+$).

1.3 Imidazolium methacrylate (Im- C_n)



Synthesis of Im- C_n was similar to Py- C_n . The purification of intermediate compound ImMA was realized by flash column chromatography on silica gel using $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}$ (12/1, v/v) as the mobile phase.

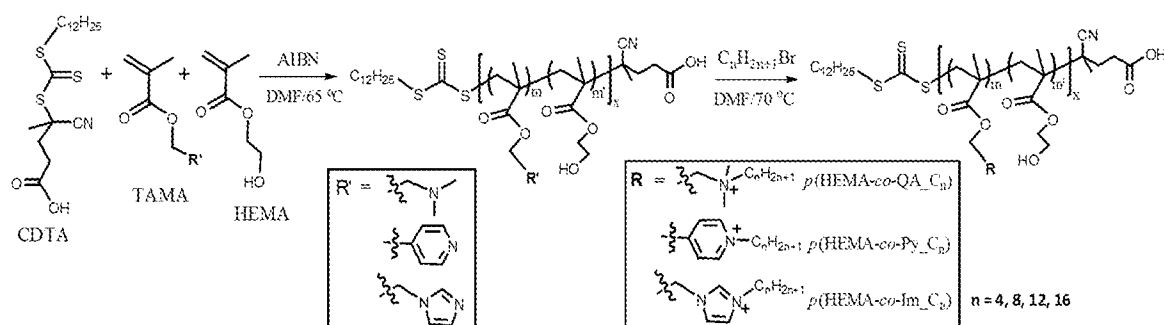
^1H NMR of characterization ImMA (400 MHz, CD_2Cl_2): δ (ppm) = 1.92 (s, 3H, $\text{CH}_2\text{C}(\text{CH}_3)\text{COO}$), 4.25 (t, 2H, $\text{COOCH}_2\text{CH}_2\text{N}$), 4.38 (t, 2H, $\text{COOCH}_2\text{CH}_2\text{N}$), 5.61 and 6.08 (s, 2H, $\text{CH}_2\text{C}(\text{CH}_3)\text{COO}$), 7.0 (d, 2H, NCHCHN), 7.49 (s, 1H, NCHN).

^1H NMR of characterization Im- C_n (400 MHz, d_6 -DMSO): δ (ppm) = 0.83 (t, 3H, $\text{N}^+\text{CH}_2\text{CH}_2(\text{CH}_2)_n\text{CH}_3$), 1.24 (m, 2nH, $\text{N}^+\text{CH}_2\text{CH}_2(\text{CH}_2)_n\text{CH}_3$), 1.74 (m, 2H, $\text{N}^+\text{CH}_2\text{CH}_2(\text{CH}_2)_n\text{CH}_3$), 1.81 (s, 3H, $\text{CH}_2\text{C}(\text{CH}_3)\text{COO}$), 4.17 (t, 2H, $\text{N}^+\text{CH}_2\text{CH}_2(\text{CH}_2)_n\text{CH}_3$), 4.44 (t, 2H, $\text{COOCH}_2\text{CH}_2\text{N}$), 4.52 (t, 2H,

$\text{COOCH}_2\text{CH}_2\text{N}$), 5.68 and 6.0 (s, 2H, $\text{CH}_2\text{C}(\text{CH}_3)\text{COO}$), 7.83 (d, 2H, NCHCHN), 9.34 (s, 1H, NCHN).

Example 2: Synthesis of PCC polymers (pPCCs)

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Three series of tertiary amine (TA)-containing polymers were synthesized by RAFT co-polymerizations of TAMA and HEMA in the presence of the chain transfer agent CDTA. In detail, CDTA (0.248 mol), TAMA (7.44 mol),
 10 HEMA (6.2 mol) and AIBN (0.0496 mol) were dissolved in 10 mL of DMF in a Schlenk flask, followed by five “Freeze-Pump-Thaw” cycles to remove the oxygen. Then the flask was filled with argon and sealed to allow polymerization at 65 °C for 12 h. After that, the flask was cooled to room temperature and the viscous solution was precipitated in a large excess of
 15 diethyl ether to obtain the product in 70% yield.

Afterwards, quaternization of the above polymers was carried out using 10-time-excess of alkyl bromides in relation to the nitrogen component. The reaction was conducted at 70°C in DMF for 2 days and finally, the reaction mixture was precipitated in a large excess of n-hexane/diethylether (3/1,
 20 v/v). To fully remove the unreacted alkyl bromides and monomers, the precipitated polymers were re-dissolved in ethanol, followed by dialysis against a solvent mixture of ethanol/acetone (10/1) for 1 week. The final products were obtained after evaporating the solvent remaining in the

dialysis bags (Cellulose membrane, cut-off molecular weight: 3.5 KD). From the ^1H NMR spectra of *p*(HEMA-*co*-QA_C8), *p*(HEMA-*co*-Py_C8) and *p*(HEMA-*co*-Im_C8) in d_6 -DMSO (data not shown), it could be concluded that the efficiency for quaternization reaction is higher than 95%

5 Example 3: Photopolymerizations

3.1 Preparation of antimicrobial polymer networks by copolymerization of PCC monomers

Homogeneous resin mixtures containing 50 mol% of UDMA, 36 mol% of TEGDMA and 14 mol% of PCC monomers were prepared by gentle
10 sonication of the components at room temperatures for 60 min. In order to initiate the polymerization under visible light, CQ (photo-initiator) and EDMAB (co-initiator) at an amount of 0.5 mol% in relation to the total amount of double bonds were added and sonication was performed for another 30 min to dissolve the photo-initiator and co-initiator. After that,
15 the viscous solutions were placed between two thin transparent glass slides which enclose a stainless steel mold (diameter: 20 mm; thickness: 0.5 mm). The photo-curing was carried out by vertical illumination of both sides of the polymerization mold at room temperature for 2 min respectively using a dental light source (Optilux 501) with an irradiance of circa
20 1000mW/cm². After photo-curing, the sample surfaces were washed with isopropanol to remove unreacted monomers.

3.2 Preparation of semi-interpenetrating polymer network incorporating pPCC (pPCC-SIPN)

In this example, we improved the biological safety of the 3D-printed polymer
25 material by incorporating pPCC in a semi-interpenetrating polymer network to obtain a pPCC-SIPN.

In light of the free radical polymerization kinetics, the rapid formation of the cross-linked network abruptly decreases the mobility of

monomers at advanced stages of conversion, resulting in the incomplete polymerization of cationic moieties. The unreacted PCC monomers as well as small oligomers remaining inside the resin may leach out over a long time and have the potential to cause cumulative toxicity to the surrounding tissue. Indeed, low levels of quaternary ammonium salt monomer were
5 detected in the supernatant of a resin sample fabricated from QA_C₁₂ by Ultra Performance Liquid Chromatography coupled to mass spectrometry (data not shown).

To avoid leaching of any unreacted cationic monomers from the resin, a novel fabrication strategy was employed. Instead of directly
10 incorporating small molecule PCC monomers into the polymer network, a high molecular weight antibacterial cationic polymer was incorporated into the resin. In this case, a semi-interpenetrating polymer network (SIPN) is formed (Figure 3b), trapping the positively charged antibacterial
15 macromolecule inside the cross-linked matrix. Such a material is realized in two steps: first, PCC monomers are converted into a PCC-containing polymer (pPCCs; Figure 3a) and then pPCCs are mixed with the frame components for photo-curing (Figure 3b). The exemplary pPCCs were synthesized by copolymerization of cationic monomers and 2-hydroxyethyl
20 methacrylate (HEMA). This co-monomer was selected because it increases the compatibility with the frame components while binding to the ester and urethane groups within the cross-linked matrix through hydrogen bonding. Before incorporation into the final resin, unreacted PCC monomers and oligomers can be removed through a simple precipitation and dialysis
25 procedure. More specifically, first homogeneous mixtures containing 40 wt% of UDMA, 40–44 wt% of GDMA and 16–20 wt% of pPCCs were prepared and then 1 mol% of CQ and EDMAB in relation to the total amount of double bonds were added, followed by sonication of the mixture for 1 h to form a clear solution. Subsequently, photo-curing was carried out as

described in section 3.1. After photo-curing, the sample surfaces were washed with isopropanol to remove the unreacted monomers.

Analysis of the polymerization kinetics showed that the incorporation of the pPCCs has no significant influence on the conversions of matrix resins. Similar to the small molecule-based PCC systems, contact-killing tests with *S. mutans* NS of three series of pPCCs-containing SIPN systems were carried out. The results (Figure 4a-b-c) indicate that all pPCC-containing SIPN exhibited contact-killing efficacies towards *S. mutans* to some extent, with the imidazolium-based SIPN exhibiting the best contact-killing effect. Interestingly, the alkyl chain length influence on the contact-killing efficacies was not as pronounced as for the small molecule-based PCC systems, especially for the imidazolium-containing SIPNs.

3.3 Polymerization kinetics

The conversion of double bonds as a function of time was determined by Fourier transform infrared spectroscopy (FTIR). Therefore, a small amount of monomer mixture was homogeneously spread between two KBr pellets to form a very thin film. The samples were irradiated for defined period of times with the dental light source (Optilux 501) and subsequently the FTIR spectra were recorded. The degree of conversion was obtained from the difference of peak areas at a wavelength of 1638cm^{-1} (C=C stretching vibration) before and after polymerization.

Example 4: Antibacterial evaluation

4.1 Determination of minimum biocidal concentration (MBC)

MBCs of PCC monomers were determined as follows: 2 mL of PCC monomer solutions were mixed with sterilized Todd-Hewitt Broth (THB) medium to yield a series of concentrations ranging from 20 mg/mL to 0.2 µg/mL PCC

monomers in sterilized test tubes. Then 10 μ L of *S. mutans* NS suspension (2×10^8 per mL) was transferred to each PCC monomer solution, followed by incubation at 37°C overnight. After that, each tube was examined for turbidity. Samples being not turbid were transferred to a swollen

- 5 Petrifilm(3M, Cat. No. 6400) and were incubated at 37°C for 48 h. MBC of a sample was defined as the minimum concentration without colonies forming units (CFUs) on the Petrifilm.

MBCs of PCC-containing polymers were determined in a different manner than PCC monomers. Due to precipitation of positively-charged
 10 polymers in THB medium, we used adhesion buffer as alternative medium to incubate a series of concentrations of polymers with 10^6 *S. mutans*/mL for 2h. The other procedures were similar to that for MBC determination of PCC monomers. The results are presented in Table 1.

15 Table 1. Minimum Biocidal Concentration (MBC) of PCC monomers and PCC-containing polymers

PCC monomers		MBC (μ g/mL)	PCC-containing polymers		MBC (μ g/mL)
QA_C _n	n=4	15,000	<i>p</i> (HEMA- <i>co</i> -QA_C _n)	n=4	125
	n=8	128		n=8	100
	n=12	4		n=12	20
	n=16	2		n=16	100
Py_C _n	n=4	> C _{sat.} *	<i>p</i> (HEMA- <i>co</i> -Py_C _n)	n=4	< 5
	n=8	100		n=8	1
	n=12	8		n=12	—**
	n=16	4		n=16	—**
Im_C _n	n=4	10,000	<i>p</i> (HEMA- <i>co</i> -Im_C _n)	n=4	< 5
	n=8	128		n=8	< 5
	n=12	10		n=12	20
	n=16	5		n=16	125

*Saturated concentration in THB medium (for Py_C₄, C_{sat.} = 4 mg/mL);

**Insoluble in water

4.2 Contact-killing tests of photocured resin compositions

Petrifilm aerobic count plates (3M, Cat. No. 6400) were used to evaluate the contact-killing activities of different formulations of photo-cured composites employing *S. mutans* NS as model strain. The experimental procedures are as follows: The top layer of the petrifilms was lifted to expose the substrate (plating surface) containing the gelling-agent and 1mL of sterilized demineralized water was added. Then the top film was slowly rolled down and a plastic “spreader” was used for even distribution of the liquid. After keeping the film at room temperature for 1h (gelling occurs), the disc (the surface was pre-cleaned using 70% alcohol) was placed in between the two layers, followed by addition of 10 μ L of *S. mutans* NS suspension (1×10^4 /mL) on the disc surface. After closing the top layer, petrifilms were kept at 37°C for 48 h, after which the number of colonies was counted. Very few CFUs were found on the UDMA/GDMA/QA_C12 (14 mol%) disc (Figure 6b), compared to the control disc only containing UDMA/GDMA (Figure 6a). To verify that the antimicrobial effect is induced solely by the surface and not by leaching of PCCs, we incubated the 3D-printed disc in PBS buffer for two days, after which we used the washing solution to culture *S. mutans* NS. As evidenced by the results presented in Figure 6c, no antimicrobial activity was detected.

4.3 CLSM observation of biofilm-inhibition

To investigate the long-lasting antibacterial properties of the 3D-printed PCC-containing resins of the invention, biofilm formation on the sample surface was followed by confocal laser scanning microscopy (CLSM). First, *S. mutans* were suspended in sterilized adhesion buffer (2 mM potassium phosphate, 50 mM potassium chloride, and 1 mM calcium chloride, pH 6.8) at a concentration of 3×10^8 /mL. Then control resin samples as well as different resin formulations containing PCCs were incubated with 3 mL of this bacterial suspension at 37°C for 5 h, followed by exchanging the buffer

with 3 mL of fresh THB medium. The samples were incubated at 37°C for another 6 days and the growth medium was exchanged every 48 h. After that, each sample surface was stained with a mixture of SYTO 9 and propidium iodide dyes (LIVE/DEAD® *BacLight*™ Bacterial Viability Kits) at room temperature for 15 min in dark for confocal microscopy observations.

A clear contact-killing effect is observed for the formulation containing 14 mol% of QA_C12 compared to a 3D-printed control disc without cationic groups. A dense biofilm formed on the control sample, in which the vast majority of bacteria remained alive after 6 days. At the same time point, only a few living bacteria and many dead bacteria were detected on the 3D-printed material containing the quaternary ammonium groups (see Figure 2c).

Example 5: 3D printing

Once the bactericidal properties of the PCC-incorporating material were established, the resins were employed for 3DP.

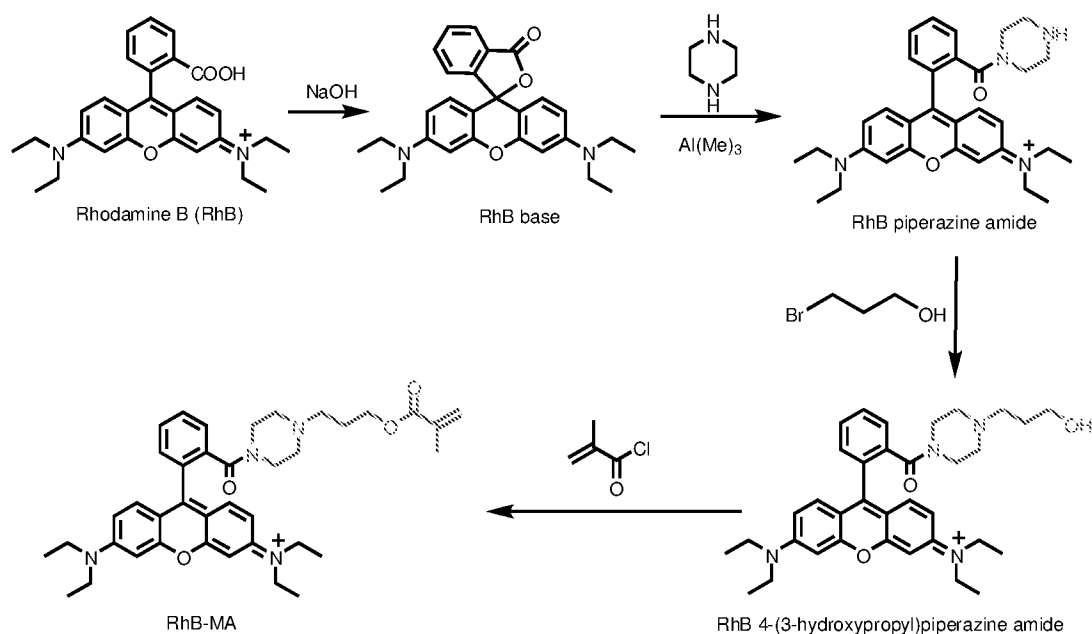
Two series of photocurable resin compositions were prepared for 3D printing. That required adjustment of the resin formulations described in section 6.1 and 6.2. To fit the laser wavelength of the SLP printer (Formlabs), 1wt% of bisacrylphosphine oxide photoinitiator (Ir819) was added to the monomer mixture, instead of CQ and EDMAB. For 3D printing, several specially designed CAD dental models (i.e. molar tooth and splint) as well as specific testing bars (i.e. thin discs for antibacterial evaluations, dumbbell-shaped specimens for tensile tests) were sliced and each slice was projected onto the bottom layer of the resin tank of the printer. After transferring the resin mixtures into the resin tank, the printing process was started and a beam of UV light draws the object onto the surface of the liquid. Once a layer was completely traced and cured, the z-stage with the substrate was moved upwards by 200 µm, covered with new

resin and the next layer was cured. The resolution of the device was approximately 300 μm in the xy-plane and 25 μm in the z-direction. After all layers were printed, the printed objects were removed from the platform and washed with isopropanol to remove the adhering resin liquid. Finally, post-printing photo-curing was carried out with models in a UV chamber for another 5h. A molar tooth model (Figure 2A, top) and a clear dental splint (Figure 2A, bottom) were successfully fabricated by subjecting a UDMA/GDMA/QA_C12 formulation to SLP 3DP. In order to test the mechanical properties as well as the contact-killing efficacies, dumbbell-shaped and disc-shaped specimens were printed, respectively.

Example 6: Leaching properties of polymers

Leaching properties of the cationic polymer out of the SIPN were investigated using the polymerizable fluorescent probe methacrylate-functionalized rhodamine B (RhB-MA).

6.1 Synthesis of RhB-MA



The modification of rhodamine B with a methacrylate group (RhB-MA) is shown in the scheme above. First, piperazine was introduced on rhodamine B through an amide linkage according to the previously published procedure [2]. Second, nucleophilic substitution reaction of RhB functionalized with piperazine and 3-bromo-1-propanol was carried out to obtain hydroxyl-functionalized RhB (RhB/OH). Specifically, 2.0 g of the RhBpiperazine amide derivative (3.9 mmol), 0.65 g of 3-Bromo-1-propanol (4.68 mmol) and 1.0 g of *N,N*-diisopropylethylamine (DIPEA, 7.8 mmol) were dissolved in 15 mL of DMF, followed by stirring at room temperatures for 24 h. After that, additional 3-bromopropanol (0.65 g, 4.68 mmol) and DIPEA (1.0 g, 7.8 mmol) were added and the resulting solution was stirred for additional 6 h. To the mixture, 100 mL of aqueous saturated NaHCO₃ solution was added and washed with three portions of EtOAc (30 mL each). Then the aqueous phase was extracted with two portions of *i*PrOH/CH₂Cl₂ (1/3 v/v, 50 mL each). The organic layer was collected, dried over anhydrous Na₂SO₄, filtered, and concentrated to afford RhB/OH (yield: 65%). Finally, RhB/OH was reacted with methacryloyl chloride to give methacrylate-functionalized RhB. Therefore, 1.0 g of RhB/OH (1.56 mmol) was dissolved in 50 mL of dry CH₂Cl₂ containing 0.19 g of TEA (1.87 mmol), followed by dropwise addition of methacryloyl chloride (0.163 g, 1.56 mmol) in 20 mL of dry CH₂Cl₂ at 0 °C. After the addition was completed, the reaction was allowed to proceed at room temperature overnight. The produced salts were removed by filtration and the filtrate was concentrated, followed by column chromatography on silica gel using CH₂Cl₂/CH₃OH (10/1, v/v) as the eluent (yield: 78%).

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6.2 Synthesis of RhB-labeled polymers

The RhB-labeled PCC polymer was prepared by in situ co-polymerization of HEMA, QA_C₁₂ and RhB-MA. For comparison, polymerizations were conducted either in presence or absence of RAFT reagents. The reaction

mixtures of the above two polymers were both designed to yield a molecular weight of 10 kDa and the polymerization procedures were chosen similar to those described in section 4. After polymerization, the product was re-dissolved in ethanol and dialyzed against distilled water until no red color could be observed in the dialysed medium. The solution in the dialysis bag was then freeze-dried to yield polymers of deep red color. ¹H NMR spectra indicated successful introduction of RhB in the polymer backbone. The RhB content within the polymer was determined by measuring the UV absorbance of the polymer solution in DMSO at 561 nm (OD_{561nm}). A calibration curve was recorded from a series of concentrations of free RhB-MA (C_{RhB-MA}) in DMSO: OD_{561nm} = 0.00236 + 110.494*C_{RhB-MA} (mg/ml).

6.3 Release from polymers

Three different formulations of prepolymer mixtures were prepared separately (Figure 3A). The portion of RhB was adjusted to 1 mol% for all formulations. Monomers of QA_C12, and HEMA were copolymerized with RhB-MA and the resulting copolymer (Figure 3B) was incorporated into a GDMA matrix. As control, RhB-MA was integrated directly into a photo-cured GDMA/QA_C12 resin. Here, we chose Ir819 as the photoinitiator due to its good efficiency of photoinitiation to the pigmented materials [3]. After dissolving the photoinitiator in monomer mixtures, they were transferred to the polymerization mould and irradiated with UV light (Intelliray-600, 150 mW/cm²) for 5 min to obtain a disc-shaped specimen with a diameter of 10 mm and a thickness of 0.5 mm.

For leaching experiments, the three discs were immersed in equal volumes of PBS buffer (10 mM, pH = 7.4) at room temperature (Figure 5). The release media was collected every week and at the same time fresh PBS buffer was added accordingly. The duration of the leaching experiment was 4 weeks and finally the fluorescent intensity of collected release media was

determined (excitation wavelength: 561 nm, emission maximum: 585 nm). The cumulative release profiles of RhB for three different formulations were determined and are shown in Figure 5c. When RhB was directly copolymerized with GDMA around 1% of the dye was released from the bulk material. In stark contrast, when RhB was prepolymerized by free radical polymerization, 10-fold less fluorophore was liberated from the SIPN (Figure 5c). These measurements indicate that leaching of cationic moieties from the resin can be significantly reduced by trapping a pre-synthesized cationic polymer inside the polymer network.

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To even further reduce liberation of cationic moieties out of the resin, a new polymerization technique was employed. Since free radical polymerization of p(HEMA-co-QAC12MA) resulted in a broad molecular weight distribution (polydispersity index (PDI) = 1.89, Mw = 10,000 g/mol) containing a significant amount of low molecular weight components, we fabricated the cationic polymer by a process called reversible addition fragmentation transfer (RAFT) polymerization³⁸. RAFT is known to yield narrowly dispersed polymers with high molecular weights, a polymer characteristic that was also achieved for p(HEMA-co-QAC12MA) (PDI = 1.16, Mw = 9800 g/mol). We found that the cationic polymer produced by the RAFT process leached out from the resin to a lesser extent compared to the cationic macromolecule fabricated by the free radical polymerization process (Figure 5c). Only 400 ppm of the fluorescent dye was detected after four weeks in the leaching tests. Without wishing to be bound by theory, this can be explained by either the decreased diffusion of the high molecular weight pPCC produced by RAFT polymerization or by covalent incorporation of the pPCC chain into the photo-cured resin.

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Example 7. Mechanical tests

Tensile tests of dumbbell-shaped specimens were carried out to compare the mechanical properties of the materials fabricated by normal curing and 3D printing. The dimensions of the test geometries were the following:

- 5 thickness: 2.0 mm; width: 4.5 mm; gauge length: 15 mm. The dumbbell-shaped specimens were clamped vertically between two holding grips and then the crosshead elongated the samples with a speed of 1 mm/min until the specimen broke. As such breaking stress and strain% were recorded. Both samples exhibited very similar mechanical properties in tensile tests.
- 10 This is reflected in the similar breaking stress and breaking elongation values (Figure 2B), indicating that CAD-sliced layers were fused together well during the layer-by-layer photo-curing.

- Subsequently, the 3D printability of pPCCs-containing resins was
- 15 investigated. In view of starting material costs, we only selected *p*(HEMA-co-QAC₁₂MA) as a model pPCC to mix with the frame components. As shown in Figure 4d, different dental models and appliances were successfully fabricated by SLP and their geometry and sizes were in accordance with the pre-determined specifications. It was found that the
- 20 compatibility of a resin composition with 3DP was dependent on the viscosity of the printing liquid. Printing was highly successful when the content of the high-viscosity UDMA was above 40wt%. Contact killing tests were then carried out with the 3D-printed resin specimens. A *p*(HEMA-co-QAC₁₂MA)-containing sample showed strong contact-killing efficacies in
- 25 relation to a control in which the cationic polymer is absent (data not shown). Again, the tensile-determined mechanical properties of 3D-printed samples were nearly the same as for materials fabricated in a polymerization mold (Figure 7).

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Claims

1. A photocurable antibacterial resin composition, comprising
 - (i) a polymer matrix comprising at least one biocompatible
5 polymerizable acrylic compound;
 - (ii) a photoinitiator system;
 - (iii) a methacrylate-based positively charged compound (PCC)
having antibacterial properties, and wherein the methacrylate-based PCC is
present in the composition as polymeric PCC (pPCC) obtained by
10 polymerization of methacrylate-modified PCC monomers.
2. Resin composition according to claim 1, wherein said biocompatible
polymerizable acrylic compound is selected from the group consisting of
mono-, di- or poly-acrylates and methacrylates.
3. Resin composition according to claim 1 or 2, wherein said polymer
15 matrix comprises diurethanedimethacrylate (DUDMA) and glycerol
dimethacrylate (GDMA), preferably at least 40wt% DUDMA and/or 20-
40wt% GDMA.
4. Resin composition according to claim 1 or 2, wherein said polymer
matrix comprises urethane di(meth)acrylate (UDMA) and one or more
20 selected from triethyleneglycol dimethacrylate (TEGDMA), tetraethylene
glycol diacrylate (E4-A), and trimethylolpropanetriacrylate (TTA),
preferably wherein the polymer matrix comprises UDMA and TEGDMA.
5. A photocurable antibacterial resin composition, comprising
 - (i) a polymer matrix comprising at least one biocompatible polymerizable
25 acrylic compound, wherein said polymer matrix comprises
diurethanedimethacrylate (DUDMA) and glycerol dimethacrylate (GDMA),
preferably at least 40wt% DUDMA and/or 20-40wt% GDMA;

(ii) a photoinitiator system;

(iii) a methacrylate-based positively charged compound (PCC) having antibacterial properties.

6. Resin composition according to any one of the preceding claims,
5 wherein said methacrylate-based PCC is a PCC modified with 2-hydroxy methacrylate (HEMA).

7. Resin composition according to any one of the preceding claims, wherein said methacrylate-based PCC is a methacrylate-based quaternary ammonium, pyridinium or imidazolium.

10 8. Resin composition according to claim 7, wherein said methacrylate-based PCC is a methacrylate-based imidazolium.

9. Resin composition according to claim 7 or 8, wherein said methacrylate-based quaternary ammonium, pyridinium or imidazolium is alkylated with an alkyl chain comprising at least 4 carbon atoms, preferably
15 at least 6, more preferably at least 8.

10. Resin composition according to any one of the preceding claims, wherein the methacrylate-based PCC is present in the composition as polymeric PCC (pPCC) obtained by reversible addition fragmentation transfer (RAFT) polymerization.

20 11. Resin composition according to any one of the preceding claims, wherein the pPCC has an average molecular weight Mw of above 2500 g/mol.

12. A method for providing an antibacterial polymer, comprising providing a resin composition according to any one of the preceding claims
25 and inducing photocuring.

13. Method according to claim 12, wherein said photocuring comprises stereolithography processing (SLP).

14. An antibacterial polymer obtainable by a method according to claim 12 or 13.

15. Polymer according to claim 14, wherein the methacrylate-based PCC is present as a polymer that is trapped inside the crosslinked polymer matrix (SIPN)

16. Polymer according to claim 14 or 15, in the form of an object selected from the group consisting of antimicrobial coatings, films, 3D printed object, consumer goods and packaging, educational object, electronics, hearing aids, sporting goods, jewelry, medical devices and toys.

17. Polymer according to claim 16, wherein said 3D printed object is a dental product.

18. Dental product according to claim 17, selected from the group consisting of dental prosthesis, artificial teeth, dentures, splints, veneers, inlays, onlays, copings, frame patterns, crowns and bridges.

19. A method for providing a 3D antimicrobial object, comprising the steps of:

a. loading a photocurable antibacterial resin composition according to any one of claims 1-11 as a liquid into a resin bath of a 3D printer based on stereolithography or other light irradiations;

b. using laser beam or light irradiation tracing out the shape of each layer of the liquid resin composition to form a polymerized solid; and

c. applying one or more successive layers of the polymerized material until an object of predetermined shape is formed.

Figure 1

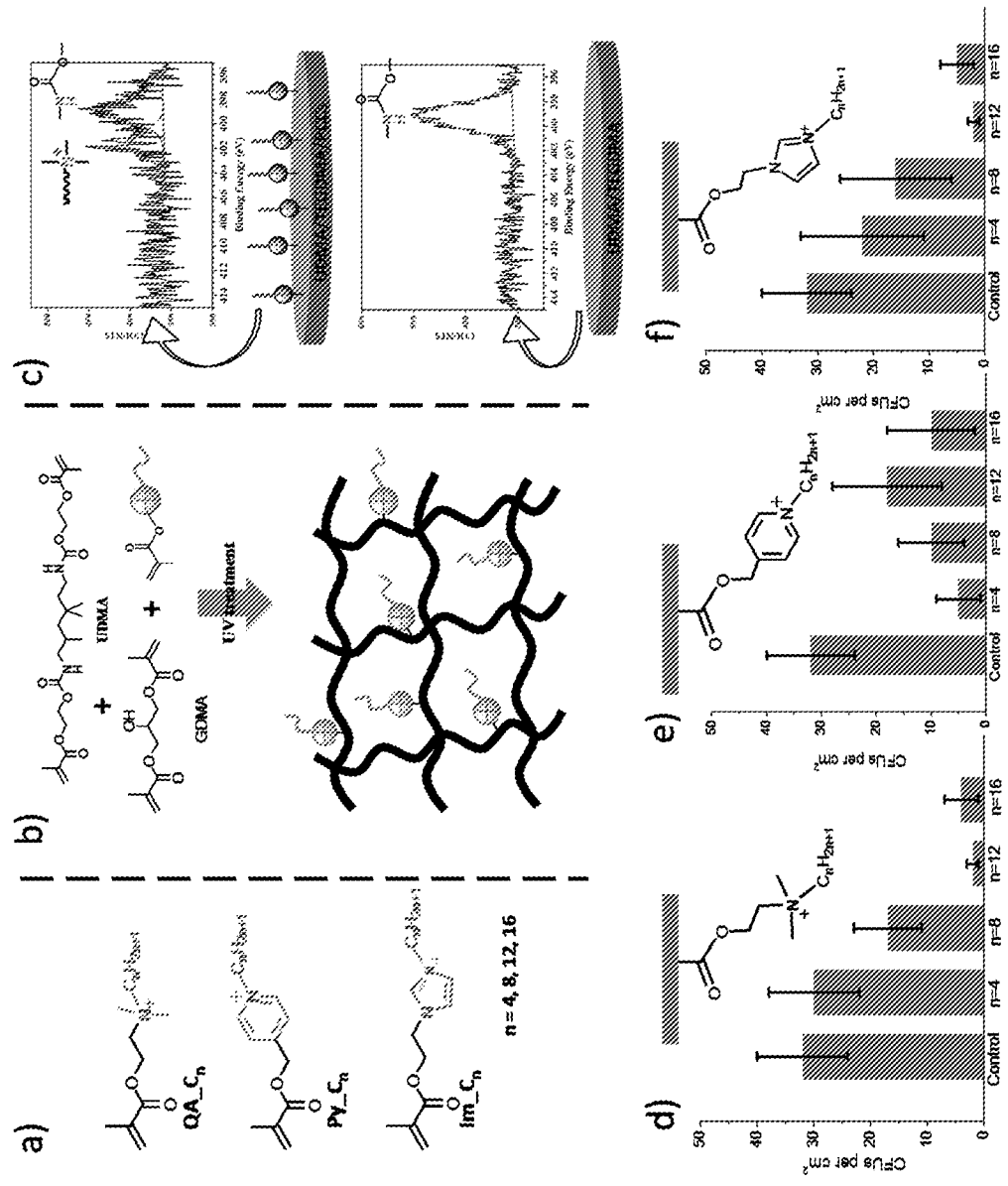


Figure 2

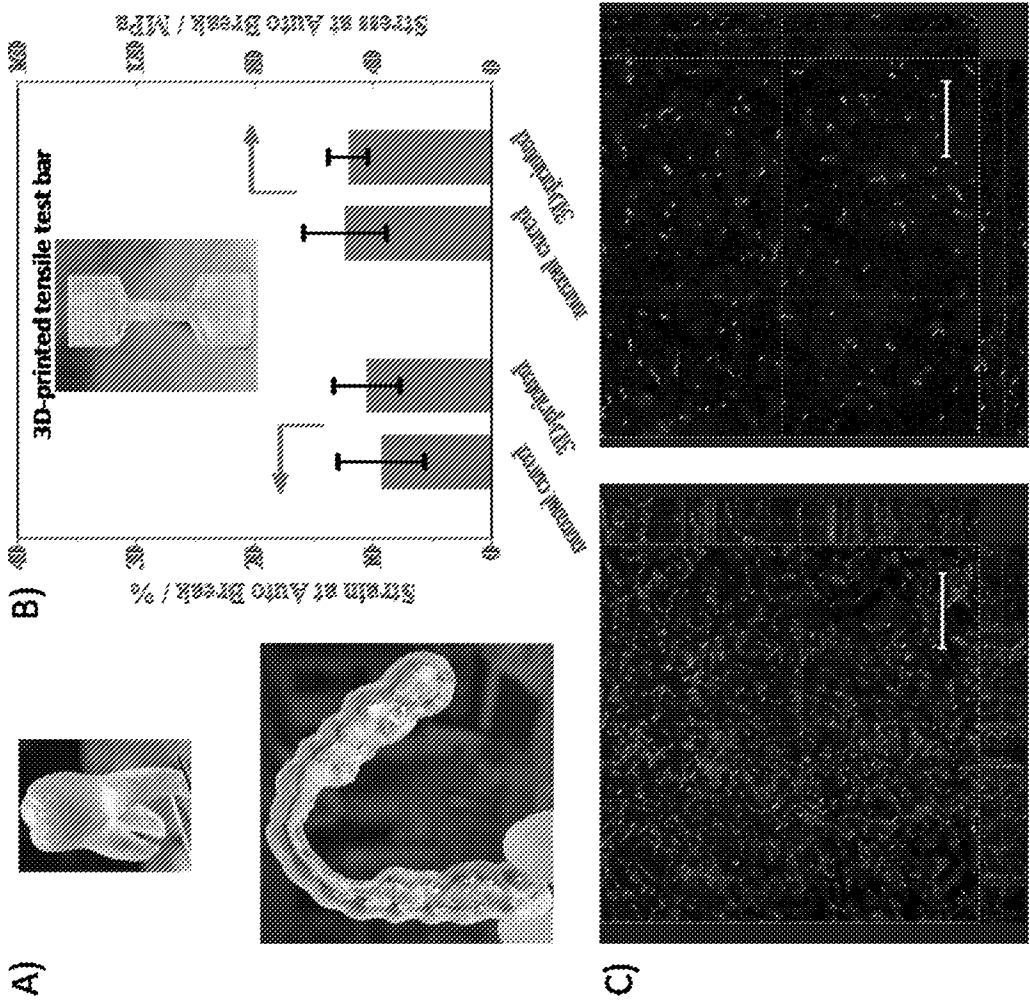
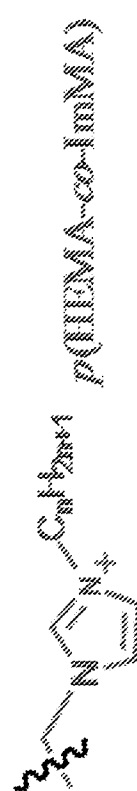
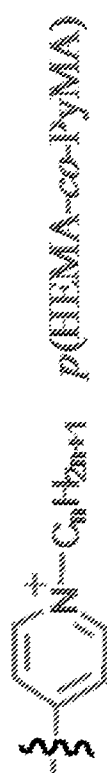
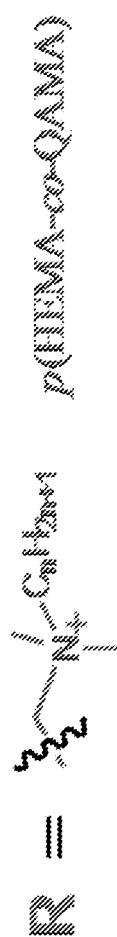
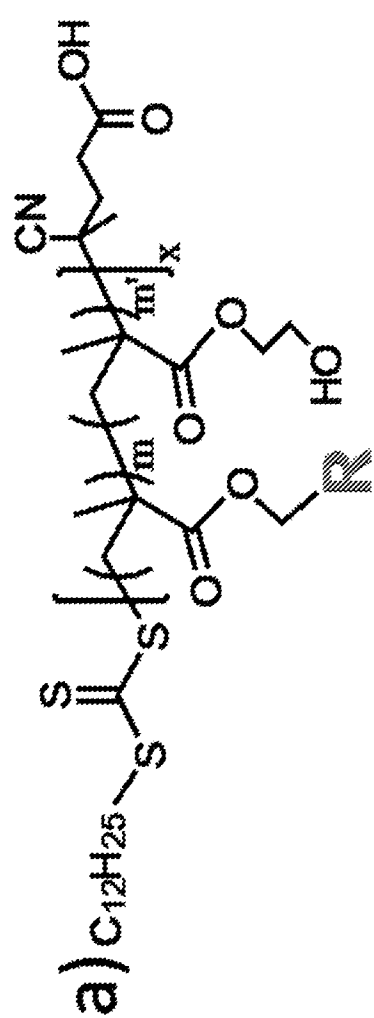


Figure 3



$n = 4, 8, 12, 16$

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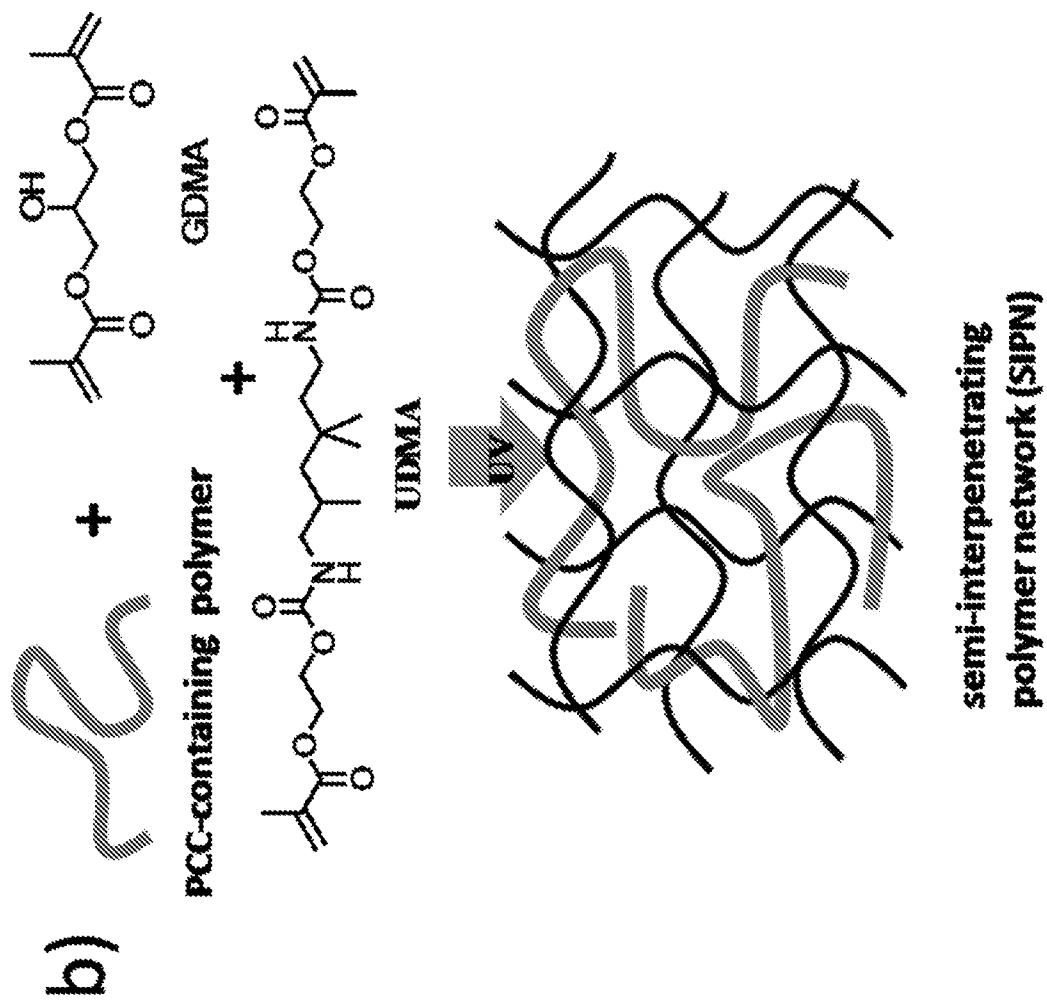
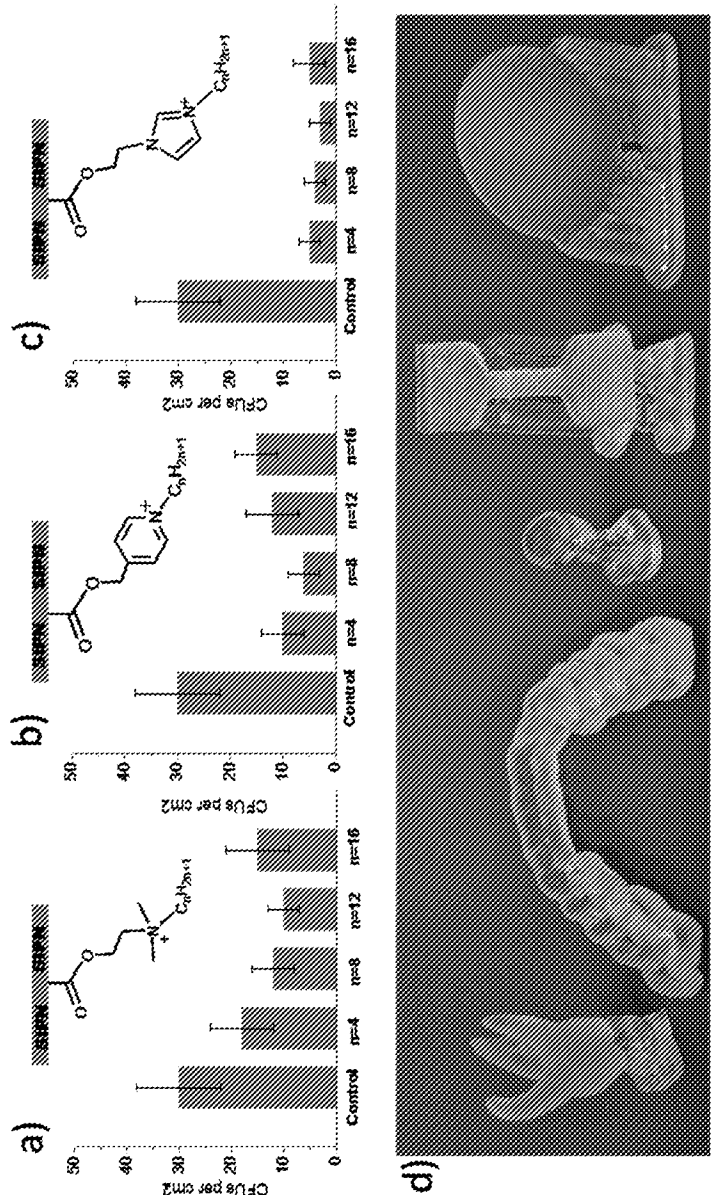
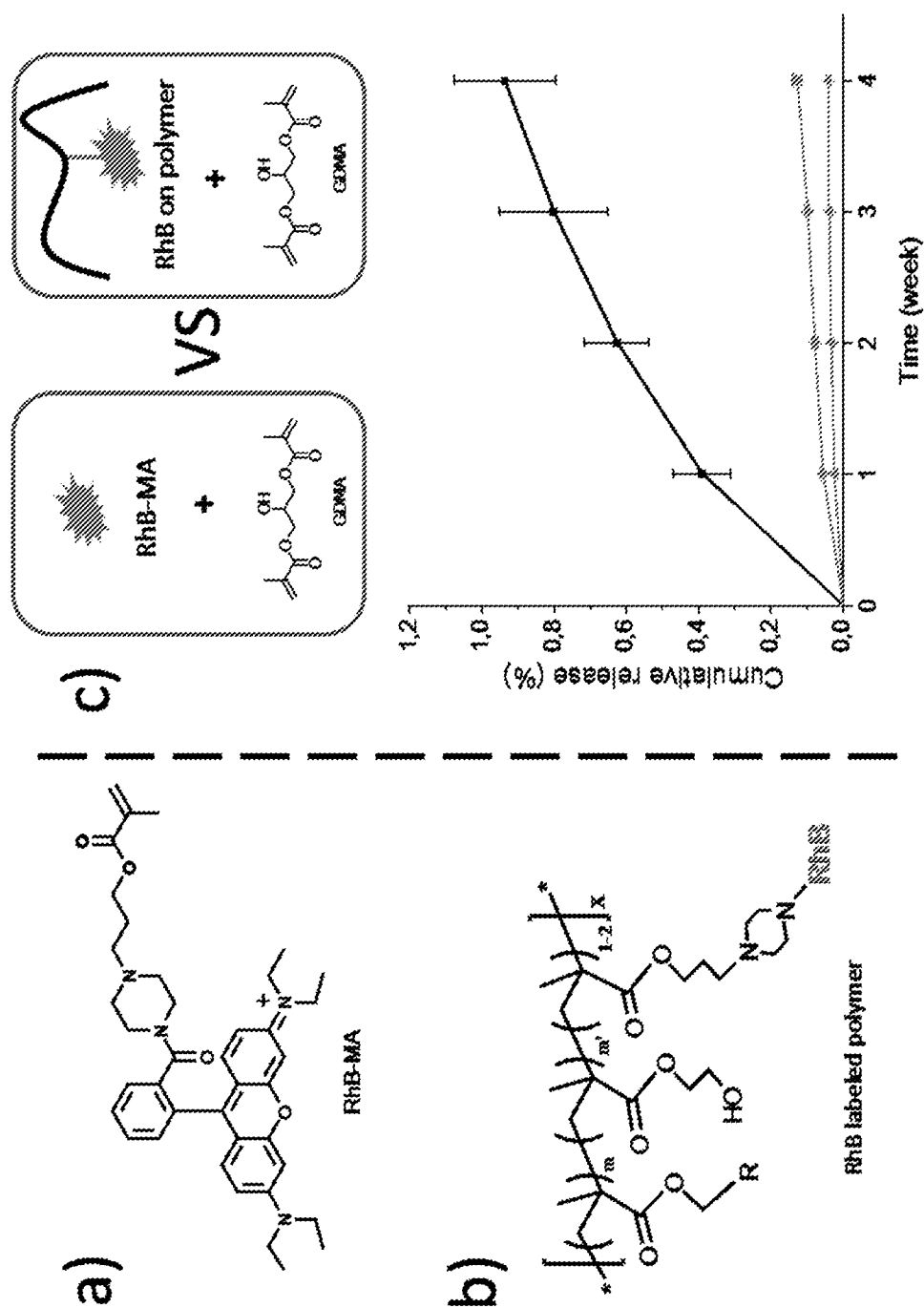


Figure 4



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Figure 5



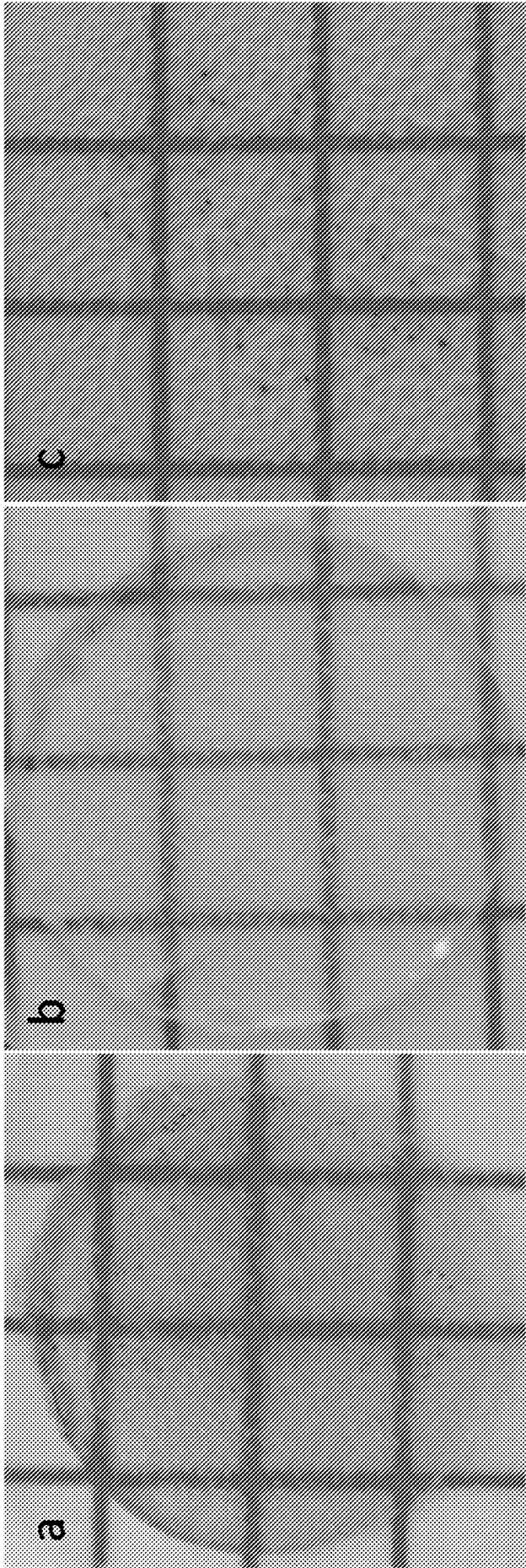


Figure 6

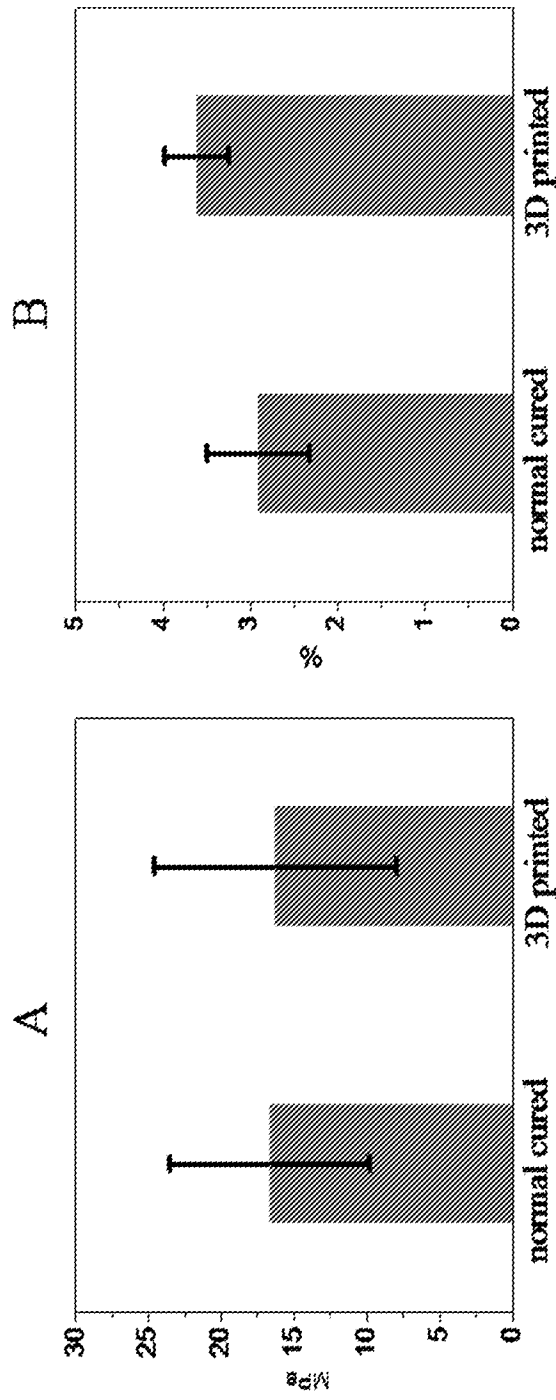


Figure 7

INTERNATIONAL SEARCH REPORT

International application No
PCT/NL2016/050339

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61C13/00 B29C67/00 A01N33/12 A01N43/40 A01N43/50
C07D233/58 C08F20/06 C08F220/06 C08L23/04

ADD.

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)

A61C B29C A01N C07D C08F C08L

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)

EPO-Internal, BIOSIS, CHEM ABS Data, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2012/177960 A1 (DENTSPLY INT INC [US]; JIN XIAOMING [US]) 27 December 2012 (2012-12-27) cited in the application	1,3,4, 7-9,11, 12,14,16
Y	figures 10-16 pages 6-19; examples paragraph [0031] claims	1-19
A	----- US 2011/256510 A1 (PASHLEY DAVID HENRY [US] ET AL) 20 October 2011 (2011-10-20) cited in the application paragraph [0185] table 3 examples claims ----- -/-	1-19



Further documents are listed in the continuation of Box C.



See patent family annex.

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"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

22 September 2016

Date of mailing of the international search report

30/09/2016

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Authorized officer

Galley, Carl

INTERNATIONAL SEARCH REPORT

International application No
PCT/NL2016/050339

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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A	----- WO 2015/061097 A1 (BASF SE [DE]; NICOLAS DEL BENE [US]; BALINT KOROSKENYI [US]; ALLISON G) 30 April 2015 (2015-04-30) cited in the application examples 5-9, 11 compounds Monomers Q1, Q2 and Q3 claims	1-19
A	----- JP 2007 302651 A (KOREA INST SCI & TECH) 22 November 2007 (2007-11-22) cited in the application examples 17, 19, 22, 24 Formula (2); page 8 claims	1-19
A	----- US 2013/195793 A1 (SCHWALM REINHOLD [DE] ET AL) 1 August 2013 (2013-08-01) cited in the application claims examples	1-19
A	----- US 2007/048249 A1 (YOUNGBLOOD JEFFREY P [US] ET AL) 1 March 2007 (2007-03-01) cited in the application figure 1	1-19
Y	----- US 2014/239527 A1 (LEE JAE SIK [US]) 28 August 2014 (2014-08-28) cited in the application tables 2, 3 claims	13,17-19
X	----- YASMINE PUPO ET AL: "Effect of a Novel Quaternary Ammonium Methacrylate Polymer (QAMP) on Adhesion and Antibacterial Properties of Dental Adhesives", INTERNATIONAL JOURNAL OF MOLECULAR SCIENCES, vol. 15, no. 5, 20 May 2014 (2014-05-20), pages 8998-9015, XP055223760, DOI: 10.3390/ijms15058998	1,2,7,9, 12,14-16
Y	abstract table 1 page 9000, line 10 ----- -/--	1-19

INTERNATIONAL SEARCH REPORT

International application No

PCT/NL2016/050339

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BR 1020 1202 6647 A2 (UNIV ESTADUAL DE PONTA GROSSA [BR]) 16 December 2014 (2014-12-16)	1,2,7,9, 12,14-16
Y	abstract claims figures -----	1-19

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Information on patent family members

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BR 102012026647 A2	16-12-2014	NONE	