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(54) Title: TRIFUNCTIONAL COATING

(57) Abstract: The invention is directed to a coating, and to an article comprising said coating and to a method of forming a coating. More in particular, the invention relates to a coating that relates to an antifouling, cell-binding and antimicrobial coating. The coating of the invention comprises a) an inner coating layer formed from an inner coating composition, the inner coating composition comprising, i) an inner polymerisable compound comprising a polymerisable group, ii) an antimicrobial agent, and iii) an initiator, and b) an outer coating layer formed from an outer coating composition, the outer coating composition comprising i) inorganic particles with a polymerisable group and a hydrophilic polymer chain attached to one or more of the inorganic particles, ii) a cell-binding moiety, iii) an initiator, and iv) optionally, an outer polymerisable compound comprising a polymerisable group.

Title: Trifunctional coating

Field

The invention is directed to a coating, to an article comprising said coating, and to a method of forming said coating. More in particular, the invention relates to a coating that may combine cell-binding and antimicrobial properties, and optionally antifouling properties.

Background

Biomedical implants are applied to restore human function, as *e.g.* in total hip replacements, central venous catheters, bone plates for trauma repair and dental implants. Still a major problem exists arising from infections around these implants, forming one of the most devastating complications following implantation of any material. Such infections may be very difficult to cure and often lead to implant replacement. Lowering the incidence and recurrence rate will improve the quality of life in this patient group and reduce hospitalisation and medication costs.

Antimicrobial coatings to biomedical implants may prevent these so-called biomaterial associated infections. Several options exist to deliver antimicrobial functionality to biomaterials. As a first option, local delivery of antimicrobials from release coatings has been successfully applied in orthopaedic implants or central venous catheters. Second, because drug releasing materials may eventually lead to bacterial resistance and drug reservoir depletion, non-adhesive coatings are being developed, which may prevent initial bacterial adhesion over long periods of time. Finally, as a third option, coatings with contact microbial activity have been developed, which directly eradicate bacteria after attachment to the implant surface. In addition to purely antimicrobial functionality, advanced antimicrobial coatings should comply with specific requirements of various applications with respect to wanted and unwanted tissue integration. In orthopaedic and

traumatology applications, for instance, cell adhesion and proliferation is essential for the successful integration of biomaterials within the host tissue, whereas urinary and central venous catheters should be prevented from cell attachment.

One of the reasons that biomaterials with antimicrobial functionalisation are not generally available yet is that each of the cases encountered in clinical practice is different and demands tailor-made solutions. For instance, in the treatment of abdominal wall hernias, four distinct infection risk scenarios demand different specific functionality to prevent implant related infections. Therefore, in order to enable tailored antimicrobial solutions, combinations of various antimicrobial and tissue integration control mechanisms are highly desired.

For instance a bi-functional coating was developed by combining a permanent contact microbial polyelectrolyte base-film with a hydrolytically degradable top-film that offers controlled release of antimicrobials (Wong, *et al.*, *Journal of the American Chemical Society* 2010, *132*(50), 17840-17848). This approach enabled the effective short term eradication of bacteria by releasing agents without losing its infection preventive characteristics in the long term. By combining antimicrobial properties with cell adhesion promoting properties, tissue integration is not hampered by the colonisation of bacteria. According to this approach, titanium alloy substrates were functionalised by chitosan, which is stated as being both antimicrobial and enhancing cell-attachment, and grafted with cell-adhesive arginine-glycine-aspartic acid (RGD) peptides by covalently binding them to the chitosan layer (Shi *et al.*, *Journal of Biomedical Materials Research Part A* 2008, *86A*(4), 865-872). The RGD peptides were able to improve the cell-attachment properties over surfaces modified with chitosan alone.

Coatings with other functionalities have also been explored. In particular, the property of suppressing or preventing biofouling is interesting. Objects made of synthetic materials in contact with water are

generally prone to undesired accumulation of biologically derived organic species, *i.e.* biofouling. Examples of biofouling phenomena include protein adsorption, bacterial adsorption and subsequent spreading, and thrombosis. This undesired accumulation has serious consequences. For example, biofouling of catheters may cause infections in patients being treated by medical professionals. In industrial processes biofouling is responsible for the clogging of filters and the undesired accumulation of organic material on surfaces.

Grafting hydrophilic polymer chains to the surface of articles made of synthetic materials has been seen as a manner to decrease or even to prevent biofouling.

A coating composition and a coating comprising hydrophilic polymer chains is known from Sophia *et al.*, *Macromolecules* 1998, *31*(15), 5059-5070. The coating is obtained by grafting hydrophilic polymer chains to a surface in order to obtain a coating comprising hydrophilic polymer chains, by using hydrophilic polymer chains with one reactive group that reacts with reactive groups on the surface. However, the thickness of the layer of grafted hydrophilic polymers may be limited. Therefore, the layer has insufficient mechanical robustness and is easily damaged, so that anti-biofouling properties are lost. A further disadvantage is that the processing is laborious, *i.e.* the chemical grafting of groups often necessitates an extra treatment of the surface to make the reaction feasible. Yet a further problem is that the anti-biofouling properties are insufficient.

Another method is using Langmuir-Blodgett technique to transfer hydrophilic chains to a surface, optionally grafting them, such as by covalently linking, afterwards, as described by Currie *et al.*, *Pure and Applied Chemistry* 1999, *71*(7), 1227-1241. However, this process is time consuming and it is only suitable for batch-wise processes and specific substrates. A further problem is that the anti-biofouling properties are not

optimal and again the mechanical robustness of such a coating is insufficient.

Yet another method is to use a cross-linked coating comprising reactive polymers. These can be either linear polymers cross-linked *in situ* by electron beam, as described by Krsko *et al.*, *Langmuir* 2003, *19*(14), 5618-5625, or star-like polymers which cross-link via *e.g.* isocyanate groups. This results in hydrogel coatings, which have protein repellent properties that are lubricious but lack mechanical robustness.

WO-A-2006/016800 discloses coating compositions and coatings comprising particles chemically grafted with reactive groups and hydrophilic polymers. These coatings may display good mechanical properties (hardness and scratch resistance), and also good anti-biofouling properties, good anti-fogging properties, good adhesion to substrates, good lubricious properties, and good optical clarity.

US-A-2014/0 228 466 describes an antimicrobial composition that is ultraviolet curable. The composition comprises an adhesive comprising an oligomer, a monomer and a photoinitiator.

WO-A-2014/070792 describes a polymer coating composition that comprises a specific aromatic dimethacrylate component.

Whereas anti-biofouling coatings display little or no non-specific binding, in some applications it is desired that such coatings also display high binding affinity towards specific biological or organic materials and/or molecules. Such bioselective coatings have a diversity of potential applications, most importantly in orthopaedic applications. In orthopaedic applications, bioselective coatings may promote cell spreading, leading to enhanced tissue integration and bone-formation.

Bioselective coatings are also useful in a number of diagnostic devices, microtiter plates, microwell plates, microarrays (such as lab-on-a-chip), immunoassays, microfluidic devices, including cell binding applications. Particular applications of bioselective coatings in diagnostic

devices include biosensors for glucose or troponin. Another potential application includes layer-on-layer systems, for example Enzyme-Linked Immuno Sorbent Assay (ELISA), in which subsequent binding to the surface-bound target biomarker is used to facilitate detection of the target biomarker. Bioselective coatings may also find application in *inter alia* diagnostic test strips, blood collection tubes and capillaries, and diabetes device management devices.

There remains a need for coatings that combine favourable properties including antimicrobial and tissue integration properties, and optionally anti-biofouling properties. An objective of the invention is to fulfil this need.

Summary

The inventors surprisingly found that this objective may, at least in part, be met by a coating wherein a hydrophilic polymer brush coating is combined with release of a microbial agent.

Accordingly, in a first aspect the invention is directed to a coating, comprising

a) an inner coating layer formed from an inner coating composition, the inner coating composition comprising,
   i) an inner polymerisable compound comprising a polymerisable group,
   ii) an antimicrobial agent, and
   iii) an initiator, and
b) an outer coating layer formed from an outer coating composition, the outer coating composition comprising
   i) inorganic particles with a polymerisable group and a hydrophilic polymer chain attached to one or more of the inorganic particles,
   ii) a cell-binding moiety,
   iii) an initiator, and

iv) optionally, an outer polymerisable compound comprising a polymerisable group.

In a further aspect the invention is directed to an article comprising a coating according to the invention.

In yet a further aspect the invention is directed to a method of forming a coating comprising the steps of

a) applying an inner coating composition on a substrate, the inner coating composition comprising
   i) an inner polymerisable compound comprising a polymerisable group,
   ii) an antimicrobial agent, and
   iii) an initiator, and
b) curing the inner coating composition by activating the initiator, thereby forming an inner coating layer,
c) applying an outer coating composition on the inner coating layer, the outer coating composition comprising:
   i) inorganic particles with a polymerisable group and a hydrophilic polymer chain attached to one or more of the inorganic particles,
   ii) a cell-binding moiety,
   iii) an initiator, and
   iv) optionally, an outer polymerisable compound comprising a polymerisable group,
d) curing the outer coating composition, thereby forming an outer coating layer.

The coating of the invention performs surprisingly well. Whereas the antimicrobial agent may inhibit cell spreading and binding, the inventors found that this effect can be at least partially compensated for by the presence of a cell-binding moiety, while allowing the coating to retain its antimicrobial efficacy.

The coating of the invention prevents bacterial adhesion and simultaneously may stimulate cell adhesion, while the cell-binding moiety allows for cell spreading.

Brief description of drawings

Figure 1: Schematic describing coupling of MBA to α-amino-ω-carboxy PEG, and subsequent coupling of GRGDS to the resulting acrylamide functional PEG via an activated ester intermediate.

Figure 2: Cumulative release curves of coatings containing chlorhexidine.

Figure 3: BSA adsorption results relative to uncoated PET tubes before chlorhexidine release.

Figure 4: BSA adsorption results during chlorhexidine release.

Figure 5: Reduction of protein adsorption of coatings containing 5, 9.5, and 19 % of GRGDS moiety.

Figure 6: Microscope image of U2OS cell spreading after 24 hours on coatings with 5.5 wt.% chlorhexidine and increasing concentrations of GRGDS moiety.

Detailed description

The coating of the invention comprises at least an inner coating layer and an outer coating layer. The terms “inner coating layer” and “outer coating layer” indicate the relative position of the layers with respect to a substrate to be coated. Accordingly, when the coating is applied on a substrate, the inner coating layer is closer to the substrate than the outer coating layer. The inner coating layer may, for example, be in direct contact with the substrate or be in contact with additional coating layers, such as one or more primer layers. The outer coating layer on the other hand may be the outermost layer of the coating, or may be overcoated with one or more

additional layers. Preferably, the inner coating layer is in direct contact with the outer coating layer.

Inner coating layer

The inner coating layer in the coating of the invention is formed from an inner coating composition.

The inner coating composition used for forming the inner coating layer comprises

i) an inner polymerisable compound comprising a polymerisable group,
ii) an antimicrobial agent, and
iii) an initiator.

The inner polymerisable compound comprises a polymerisable group. Preferably, the polymerisable group of the inner polymerisable compound comprises a (meth)acrylic acid, (meth)acrylate, or (meth)acrylamide group. The inner polymerisable compound can suitably comprise an acrylic acid, dextran acrylate, dextran methacrylate, dextran glycidyl methacrylate, methacrylate functionalised hyaluronic acid, or acrylate functionalised hyaluronic acid. In a preferred embodiment, the inner polymerisable compound comprises an acrylic acid.

The polymerisable compound may comprise an inner cross-linker. The inner cross-linker can form cross-links in the inner coating layer by forming cross-links with either or both of itself or other compounds that comprise the inner polymerisable compound. The inner cross-linker may, for example, comprise one or more selected from the group consisting of poly(ethylene glycol)-diacrylate (PEGDA), poly(ethylene glycol)-dimethacrylate (PEGDMA), poly(ethylene glycol)-diacrylamide (PEGDAA), poly(ethylene glycol)-dimethacrylamide (PEGDMAA), glycerol dimethacrylate, glycerol 1,3-diglycerolate diacrylate, sorbitol acrylate, and derivatives thereof. Preferably, the inner cross-linker comprises one or more selected from the group consisting of poly(ethylene glycol)-diacrylate,

poly(ethylene glycol)-dimethacrylate, poly(ethylene glycol)-diacrylamide and poly(ethylene glycol)-dimethacrylamide. Good results have been obtained with poly(ethylene glycol)-diacrylamide as the inner cross-linker.

The inner cross-linker suitably comprises a hydrophilic moiety, such as a poly(ethylene glycol) moiety. A poly(ethylene glycol) moiety is a polymer of ethylene glycol repeating units. The poly(ethylene glycol) moiety of the inner cross-linker can have n repeating units of ethylene glycol, wherein n is an integer value from 0 to 10 000, for example from 1 to 5000, or from 1 to 1000, or from 1 to 100.

The inner polymerisable compound used in the inner coating composition comprises one or more polymerisable groups. Polymerisable groups may react to form a cross-linked phase so to form a coating. It is possible that a single species of reactive groups is used, able to mutually react, for example in a homopolymerisation reaction. Examples of such reactive groups include acrylate and methacrylate groups. Another possibility is that a mixture of groups is used, for example groups that are able to react in a copolymerisation reaction. Examples of such groups include carboxylic acids and/or carboxylic anhydrides combined with epoxies, acids combined with hydroxy compounds, especially 2-hydroxyalkylamides, amines combined with isocyanates, for example blocked isocyanate, uretdione or carbodiimide, epoxies combined with amines or with dicyandiamides, hydrazinamides combined with isocyanates, hydroxy compounds combined with isocyanates, for example blocked isocyanate, uretdione or carbodiimide, hydroxy compounds combined with anhydrides, hydroxy compounds combined with (etherified) methylolamide ("amino-resins"), thiols combined with isocyanates, thiols combined with acrylates or other vinyl species (optionally radical initiated), acetoacetate combined with acrylates, and when cationic cross-linking is used epoxy compounds with epoxy or hydroxy compounds. Addition reactions such as 2 + 2 photo cycloaddition and 4 + 2 thermal additions are also possible.

Suitably, the inner polymerisable compound comprises a hydrophilic polymer, preferably poly(alkylene oxide) or polyvinylpyrrolidone.

The amount of the inner polymerisable compound in the inner coating composition can range from 50-99 % by total dry weight of the inner coating composition, such as 60-99 %, or 65-95 %. The term "dry weight of the coating composition" as used in this application is meant to refer to the weight of the coating composition excluding any solvents. The inner polymerisable compound is typically the major component in the inner coating composition.

The inner coating composition further comprises an antimicrobial agent. An antimicrobial agent is an antibiotic, antimicrobial, antiseptic and/or antifungal compound. Preferably, the antimicrobial agent is an antimicrobial compound and/or an antiseptic compound. The antimicrobial agent may provide the coating of the invention with an antimicrobial functionality.

Many different antimicrobial agents can suitably be used in the coating of the invention. The antimicrobial agent can comprise one or more of a tetracycline, a biguanide (including bisbiguanides), elemental silver such as silver nanoparticles, silver oxide, silver salts such as silver nitrate, silver sulphadiazine, siver zeolites, triclosan, antifolates, aminoglycosides, carbapenems, cephalosporins, fluoroquinolines, glycopeptides, tuberculostatics, macrolides, monobactams, oxazolidinones, penicillin, sulphonamide, and/or their salts. In a preferred embodiment, the antimicrobial agent comprises silver nanoparticles, ionic silver and/or a biguanide.

The antimicrobial agent can comprise one or more of chlorhexidine, alexidine, methylisothiazolone (2-methylisothiazolone hydrochloride), thymol (5-methyl-2-sopropyl phenol), α-terpineol (α-α-4-trimethyl-3-cyclohexine-1-methanol), cetylpyridinium chloride

(1-hexadecylpyridnium chloride), and chloroxylenol (4-chloro, 3,5-dimethyl phenol).

In an embodiment, the antimicrobial agent is cytotoxic. In a preferred embodiment, the antimicrobial agent comprises chlorhexidine. Included in the definition of chlorhexidine are pharmaceutically acceptable salts of chlorhexidine. Chlorhexidine, such as chlorhexidine gluconate or chlorhexidine acetate, is a biguanide with a very rapid bactericidal activity against a broad range of microorganisms, including gram-positive bacteria (such as *Staphylococci, Enterococcus* species), gram-negative bacteria (such as *Escherichia coli* and *Pseudomonas aeruginosa*) and *Candida* species. Chlorhexidine causes disruption of microbial cell membranes and precipitation of cellular contents, and its effectiveness is not affected by the presence of organic matter, such a blood. Chlorhexidine is generally not stable for a significant amount of time in a coating composition at a pH of 8 or more.

The antimicrobial agent can be used individually or in combinations of two or more to obtain a synergistic effect. Some examples of combinations of antimicrobial agents include a mixture of chlorhexidine, methylisothiazolone and α-terpineol; thymol and chloroxylenol; thymol and methylisothiazolone; chlorhexidine and cetylpyridinium chloride; chlorhexidine and chloroxylenol; chlorhexidine with silver, such as silver nanoparticles or ionic silver; chlorhexidine with an antibiotic; or chlorhexidine, methylisothiazolone and thymol. These combinations may provide a broad spectrum of activity against a wide variety of organisms. However, other combinations of antimicrobial agents may be applied as well.

The antimicrobial agent, depending on its composition, may be capable of forming a complex with a complexing moiety. The complexing moiety is preferably charged, and in such case is a complexing ion. In the case that the complexing moiety is a complexing ion, the complexing ion will

have a charge opposite to that of the antimicrobial agent. In an embodiment, the complexing moiety is (meth)acrylic acid, preferably acrylic acid.

Preferably, the complexing moiety forms all or part of the inner polymerisable compound. Thus, preferably, the complexing moiety may be covalently bound to the inner polymerisable compound. In the case that the antimicrobial agent is chlorhexidine, it is preferred that the inner coating composition comprises a complexing moiety and that the complexing moiety is covalently bound to the inner polymerisable compound.

In an embodiment, the antimicrobial agent is entrapped in the inner coating layer. In the case that the antimicrobial agent comprises silver, such as silver nanoparticles or ionic silver, it is preferred that the antimicrobial agent is entrapped in the inner coating layer.

In an embodiment, the antimicrobial agent forms a complex with a complexing moiety and the complexing moiety is covalently bound to the inner polymerisable compound, or wherein the antimicrobial agent is entrapped in the inner coating layer.

The amount of the antimicrobial agent in the inner coating composition can be 0.05-15 % by total dry weight of the inner coating composition, such as 0.01-12 %, or 1.0-10 %. Typically, the amount of each antimicrobial agent used is sufficient to form an effective concentration to inhibit the growth of bacterial or fungal organisms for the desired coating application.

The inner coating composition further comprises an initiator to initiate (or activate) a polymerisation reaction (*viz.* a polymerisation initiator). The amount of initiator may vary between wide ranges. A suitable amount of initiator is for example 0.01-6 % by total dry weight (*i.e.* excluding solvents) of the compounds that take part in the polymerisation reaction. Normally, this approximately amounts to 0.01-6 % by total dry

weight of the inner coating composition, such 0.01-5 %, or 0.02-4 %, based on the total dry weight of the inner coating composition.

Some examples of possible initiators include benzophenone, acetophenone, benzil, benzoin, hydroxyalkylphenone, phenyl cyclohexyl ketone, anthraquinone, thioxanthone, triazine, azido, aziridine, and fluorenone derivatives. When polymerising by ultraviolet (UV) radiation, the mixture preferably comprises one or more UV photoinitiators.

Any polymerisation method that may cause the polymerisable groups to react and so to form the polymerised phase so that the inner coating is formed is suitable to be used. Suitable ways to initiate polymerisation are, for example, electron beam radiation, electromagnetic radiation (UV, visible and Near IR), thermally and by adding moisture, in case moisture curable compounds are used. In a preferred embodiment polymerisation is achieved by UV-radiation. The UV polymerisation may take place through a free radical mechanism, through a cationic mechanism, or a combination thereof. In another preferred embodiment, the polymerisation is achieved thermally. Also, combinations of different cure methods are possible. Preferably, the polymerisation results in cross-linking.

The inner coating may have any desired thickness depending on the application envisaged. Typically, the inner coating can have a thickness ranging between 50 nm to tens of micrometers, *e.g.* 50-100 000 nm, preferably 100-10 000 nm.

Outer coating layer

The outer coating layer in the coating of the invention is formed from an outer coating composition.

The outer coating composition used for forming the outer coating layer comprises

i) inorganic particles with a polymerisable group and a hydrophilic polymer chain attached to one or more of the inorganic particles,
ii) a cell-binding moiety,
iii) an initiator, and
iv) optionally, an outer polymerisable compound comprising a polymerisable group,

A wide range of possible inorganic particles can be used in the outer coating composition. Examples of suitable inorganic particles include particles that comprise SiO_2, TiO_2, ZnO_2, SnO_2, Am-SnO_2, ZrO_2, Sb-SnO_2, Al_2O_3, Au and/or Ag. In addition to the inorganic particles, the outer coating composition can comprise organic particles. Examples of possible organic particles are carbon nanotubes or carbon nanospheres.

Preferably, the average largest diameter of the particles is 10 μm or less, preferably 1 μm or less. Still more preferably, the average largest diameter of the particles is 100 nm or less, still more preferably 50 nm or less. It is also possible with particles of these very small diameters to provide a transparent coating.

In the case of spherical particles there is only one diameter to consider, so that the diameter is equal to the smallest diameter and the largest diameter. For non-spherical particles (for example rods and platelets) the largest diameter is measured as the largest straight line drawn across the particle. Methods for determining the particle dimensions include optical microscopy, scanning microscopy and atomic force microscopy (AFM). If a microscopy method is used, the dimensions of 100 randomly chosen particles are measured and the average is calculated providing the average diameter.

Attached to one or more of the inorganic particles are a polymerisable group and a hydrophilic polymer chain.

Polymerisable groups include each of the polymerisable groups mentioned hereinabove for the inner polymerisable compound.

The term "hydrophilic polymer chain" as used in this application refers to a polymer chain that dissolves in water at a temperature (or at least one temperature) between 0 and 100 °C. Preferably, a polymer is used that dissolves in water in a temperature of 20-40 °C. The hydrophilic polymer preferably dissolves for 0.1 gram or more per litre of water, more preferably for 0.5 grams or more per litre, most preferably for 1.0 gram or more per litre. The solubility of the polymer chains in water is determined by the polymer chain not comprising groups for possible grafting the polymer chains or any other group that is attached to the polymer after the polymerisation, for example an ionic group. Preferably, the solubility is determined in water having a pH of 3-10, more preferably 5.5-9, most preferably having a pH of about 7.

The hydrophilic polymer chain may comprise one monomer species (homopolymer), or more species (copolymer) arranged in a random manner or in ordered blocks.

Preferably, the hydrophilic polymer chain comprises poly(alkylene oxide). The hydrophilic polymer chain may suitably comprises monomer units of ethylene oxide, (meth)acrylic acid, (meth)acrylamide, vinylpyrrolidone, 2-hydroxyethyl(meth)acrylate, phosphorylcholine, glycidyl(meth)acrylate, betaines, or saccharides. Other monomer units include monomeric substituents of polyoxazolines, hydroxyethyl acrylate based polymers, oligo(ethylene oxide) acrylates, and oligo(ethylene oxide) methacrylates. Preferably, the hydrophilic polymer chain comprises monomer units of ethylene oxide or vinylpyrrolidone. One of the typical advantages that the outer coating layer imparts to the coated object is anti-biofouling properties of the coating, resulting from the hydrophilicity of the polymer chain. These properties may increase with increasing concentration and length of hydrophilic polymer chain at the surface of the coating.

Preferably, the hydrophilic polymer chains comprise an average of 5 monomeric units or more. More preferably, the hydrophilic polymer chains comprise an average of 7 monomeric units or more, still more preferably the hydrophilic polymer chains comprise an average of 10 monomeric units or more, most preferably the hydrophilic polymer chains comprise an average of 15 monomeric units or more.

The polymerisable group may be present on the hydrophilic polymer chains or may be otherwise attached to the inorganic particles, such as by way of a non-hydrophilic polymer chain. Preferably, 20 % or more by total weight of the hydrophilic polymer chains do not comprise such a polymerisable group. More preferably, 50 % or more, still more preferably 80 % or more of the hydrophilic polymer chains do not comprise such a polymerisable group. In an embodiment, the hydrophilic polymer chains do not comprise any of such polymerisable groups.

Groups for grafting the hydrophilic polymer chains and compounds comprising the polymerisable group to the inorganic particles may comprise all groups known in the art for grafting. Some non-limiting examples include (trialkoxy)silanes, thiols, amines, silane hydrides. In such cases, due to the grafting reaction, the hydrophilic polymer chains and the compounds comprising the polymerisable group are covalently bound to the surface of the particles. It is possible that the hydrophilic polymers and the compounds comprising the polymerisable group comprise more than one group for grafting per molecule. More preferably, the hydrophilic polymers and the compounds comprising the polymerisable group have on average one group for grafting per molecule. In case of the hydrophilic polymer the group for grafting is preferably a reactive end-group attached to the chain of the hydrophilic polymer.

The amount of the compounds attached to one or more of the inorganic particles in the outer coating composition can be 5-60 % by total dry weight of the inorganic particles with a polymerisable group and a

hydrophilic polymer chain attached to one or more of the inorganic particles, such as 10-50 %, or 15-40 %.

The amount of the inorganic particles with a polymerisable group and a hydrophilic polymer chain attached to one or more of the inorganic particles in the outer coating composition can be 1-30 % by total weight of the outer coating composition (including solvent), such as 2-25 %, or 3-20 %. The amount of the inorganic particles with a polymerisable group and a hydrophilic polymer chain attached to one or more of the inorganic particles in the outer coating composition can be from 40-95 % by total dry weight of the outer coating composition, such as 45-90 %, or 60-85 %.

The outer coating composition further comprises a cell-binding moiety.

The cell-binding moiety can be integrated into the coating by, for example, participating in the polymerisation reaction of the polymerisable groups that form the polymerised phase, or they can be grafted onto the particles in a manner analogous to the grafting of the hydrophilic polymer chains and the polymerisable groups.

The cell-binding moiety comprises one or more cell-binding groups, for example at least two or three cell-binding groups. The cell-binding moiety may comprise at least one of an optional linking group and an optional spacer group. Each of foregoing groups may serve a different purpose in the context of the coating composition, but it should be recognised that a single cell-binding group may serve multiple purposes. For example, a cell-binding moiety may have cell-binding group that serves as both a linking group and a spacer group. The cell-binding moiety may have one or more linking groups or no linking groups per molecule, one or more cell-binding groups per molecule, and optionally one or more spacer groups per molecule.

The cell-binding group of the cell-binding moiety is a functional group capable of binding to a cell, preferably with high selectivity and high

specificity. Typically, the cell-binding group can comprise an adhesive peptide fragment. Several such adhesive peptide fragments are known in the art. A particular peptide fragment can be tested for its binding ability or adhesive capacity according to standard techniques. Examples of such peptide sequences include but are not limited to: Arg-Gly-Asp (RGD)-containing peptide sequences; Tyr-Ile-Gly-Ser-Arg (YIGSR)-containing peptide sequences; and/or Ile-Lys-Val-Ala-Val (IKVAV)-containing peptide sequences.

Arg-Gly-Asp (RGD)-containing peptide sequences are widely recognised as cell recognition motifs. RGD peptides do not only trigger cell adhesion effectively but can also be used to address selectively certain cell lines and elicit specific cell responses. Further details about different RGD-containing peptides that can be used in this invention and their specific properties are described in Hersel *et al.*, *Biomaterials* 2003, *24*(24), 4385-4415.

Examples of RGD-containing peptide sequences that could be used in the present invention include but are not limited to: RGD, RGDS, GRGDS, GRGD, YRGDS, YRGDG, YGRGD, GRGDSP, GRGDSG, GRGDSY, GRGDSPK, CGRGDSY, GCGYGRGDSPG, and RGDSP ASSKP.

In one preferred embodiment of the present invention, the peptide sequence is Gly-Arg-Gly-Asp-Ser (GRGDS).

Tyr-Ile-Gly-Ser-Arg (YIGSR)-containing peptide sequences are found on the B1 chain of laminin, promotes epithelial cell attachment (Graf *et al.*, *Biochemistry* 1987, *26*(22), 6896-6900).

Ile-Lys-Val-Ala-Val (IKVAV)-containing peptide sequences are found on the A chain of laminin, and have been reported to promote neurite outgrowth (Tashiro *et al.*, *Journal of Biological Chemistry* 1989, *264*(27), 16174-16182).

The cell-binding group can comprise repeating peptide sequences (peptide monomers). The repeating peptide sequences may be homopolymers

consisting of a single repeating peptide monomer or alternatively may be heteropolymers consisting of two or more different repeating peptide monomers or subunits. In general the cell-binding group may consist of 2-100 peptide monomers, usually 2-50, preferably 3-15. Each peptide monomer may range in length from 2-40 amino acid residues, usually 2-30, preferably 2-10.

The peptide monomers may be chemically synthesised or produced by means of recombinant genetics. Similarly, the cell-binding groups comprising repeating peptide sequences may be produced by chemically linking peptide monomers together or alternatively they can be recombinantly expressed.

In one specific embodiment of the invention, the cell-binding group comprises repeating peptide sequences of RGD-containing peptide sequences.

The optional linking group is a functional group capable of participating in either a grafting reaction with the particles of the outer coating composition, or a polymerisation reaction with the polymerisable groups grafted onto the particles. In one embodiment, the linker group reacts in a cross-linking reaction with the polymerisable groups such that the cell-binding moiety becomes part of the cross-linked phase. The linking group may be at least one functional group selected from: acrylates, methacrylates, epoxies, alcohols, carboxylic acids, carboxylic anhydrides, amines, amides, acrylamides, hydroxyalkylamides, dicyandiamides, hydrazinamides, isocyanates, carbodiimides, anhydrides, thiols, acyl groups, vinyl groups, and any of the functional groups that may constitute the polymerisable groups of the outer coating composition as described above. In certain embodiments, the linking group is a monoacrylamide group. In other embodiments, the linking group is a diacrylamide group.

The optional spacer group may be provided in the cell-binding moiety in order to increase the separation between the linker group and the

cell-binding group. In one embodiment, inclusion of the spacer group increases the likelihood that cell-binding group is present at the surface of the coating. In certain embodiments, the cell-binding group is non-reactive and is compatible with the hydrophilic polymer chains grafted to the particles of the outer coating composition. Accordingly, the spacer group may be polymeric in nature; for example the spacer group may comprise repeating monomer units of ethylene oxide, propylene oxide, (meth)acrylic acid, vinylpyrrolidone, 2-hydroxyethyl(meth)acrylate, phosphorylcholine derivatives, glycidyl (meth)acrylate, heparins or saccharides.

The amount of the cell-binding moiety in the outer coating composition can be 1-50 % by total dry weight of the outer coating composition, such as 5-40 %, or 8-30 %.

The outer coating composition further comprises an initiator to initiate (or activate) a cross-linking reaction (*viz.* a polymerisation initiator). The amount of initiator may vary between wide ranges. A suitable amount of initiator is for example 0.01-6 % by total dry weight of the compounds that take part in the polymerisation reaction. Normally, this approximately amounts to 0.01-6 % by total dry weight of the outer coating composition, such 0.01-5 %, or 0.02-4 %, based on the total dry weight of the outer coating composition.

Some examples of possible initiators include benzophenone, acetophenone, benzil, benzoin, hydroxyalkylphenone, phenyl cyclohexyl ketone, anthraquinone, thioxanthone, triazine, and fluorenone derivatives. When polymerising by ultraviolet (UV) radiation, the mixture preferably comprises one or more UV photoinitiators.

Optionally, the outer coating composition further comprises an outer polymerisable compound that comprises a polymerisable group. The outer polymerisable compound comprising a polymerisable group can be chosen from the inner polymerisable compounds comprising a polymerisable group which are defined hereinabove.

The amount of the optional outer polymerisable compound in the outer coating composition can range from 0-40 % by total dry weight of the outer coating composition, such as 1-30 %, or 1-20 %.

Any polymerisation method that may cause the polymerisable groups to react and so to form the polymerised phase so that the outer coating layer is formed is suitable to be used. Suitable ways to initiate polymerisation are for example electron beam radiation, electromagnetic radiation (UV, visible and Near IR), thermally and by adding moisture, in case moisture curable compounds are used. In a preferred embodiment, polymerisation is achieved by UV-radiation. The UV cross-linking may take place through a free radical mechanism, through a cationic mechanism, or a combination thereof. In another preferred embodiment, the polymerisation is achieved thermally. Also, combinations of different cure methods are possible. Preferably, the polymerisation results in cross-linking.

The outer coating layer may have any desired thickness depending on the application envisaged. Typically, the outer coating layer can have a thickness ranging between 50 nm to tens of micrometers, *e.g.* 50-100 000 nm, preferably 100-10 000 nm.

The outer coating layer can have an average porosity of 0.1-5 % by volume of the outer coating layer, for example 0.2-4.5 %, 0.5-4 % or 1-3 %. This may allow for good diffusion of the antimicrobial agent from the inner coating trough the outer coating layer.

Additives

The inner and/or outer coating compositions can further comprise a number of additional components.

The inner and/or outer coating compositions may, for instance, comprise one or more solvents. In principle, a wide variety of solvents may be used. A solvent in the outer coating composition preferably has the ability to form stable suspensions of the particles grafted with the

polymerisable groups and the hydrophilic polymer chains, in order to obtain good quality coatings, *i.e.* after evaporation of the solvent. The particles typically are added to the mixture in the form of a suspension. The same solvent as used in the suspension may be used to adjust the mixture so that it has the desired properties. However, other solvents may also be used.

Examples of solvents that may be suitable are alcohols, ketones, and esters. Further examples of solvents are 1,4-dioxane, acetone, acetonitrile, chlorophenol, cyclohexane, cyclohexanone, cyclopentanone, diethyl acetate, diethyl ketone, dimethyl carbonate, dimethylformamide, dimethylsulphoxide, ethanol, ethyl acetate, *m*-cresol, mono- and di-alkyl substituted glycols, *N*,*N*-dimethylacetamide, *p*-chlorophenol, 1,2-propanediol, 1-pentanol, 1-propanol, 2-hexanone, 2-methoxyethanol, 2-methyl-2-propanol, 2-octanone, 2-propanol, 3-pentanone, 4-methyl-2-pentanone, hexafluoroisopropanol, methanol, methyl acetate, methyl acetoacetate, methyl ethyl ketone, methyl propyl ketone, *n*-methylpyrrolidone-2, *n*-pentyl acetate, phenol, tetrafluoro-*n*-propanol, tetrafluoroisopropanol, tetrahydrofuran, toluene, xylene and water. Halogenated solvents (such as dichloromethane and chloroform) and hydrocarbons (such as hexanes and cyclohexanes), may also be suitable. Preferably, methanol, methyl ethyl ketone or isopropanol are used. In a more preferred embodiment, mixtures of one or more organic solvents with water are used. In certain embodiments water is used as solvent, or a mixture of at least one alcohol and water is used as solvent.

The inner coating composition can further comprise one or more adhesion promoters, *i.e.* compounds that increase the adhesion of the coating to the substrate. These may be for example silane acrylate compounds for usage of acrylate-containing coatings on glass. The person skilled in the art will be able to select a suitable adhesion promoter for the desired substrate.

The inner and/or outer coating compositions can further comprise one or more species that diffuse out of the coating during usage. Such species may be used for lubricity, adhesion purposes or comprise therapeutic species. Examples of such species are for instance but not limited to heparin, vitamins, anti-inflammatory agents, antimicrobial functionalities such as quaternary ammonium ions, peptide sequences, halogen labile species, and biomolecule receptor sites.

Optional intermediate layer

Optionally, an intermediate layer may be applied between the inner coating layer and the outer coating layer. This intermediate layer can serve to smooth the transitions in mechanical properties between the inner coating layer and the outer coating layer. The optional intermediate layer preferably has a thickness in the range of 50-10 000 nm, such as 100-5000 nm.

The optional intermediate layer preferably has sufficient porosity to allow diffusion of the antimicrobial agent from the inner coating layer through the intermediate layer to the outer coating layer. The optional intermediate layer can, for example, have an average porosity of 0.1-5 % by volume of the intermediate layer, for example 0.2-4.5 %, 0.5-4 % or 1-3 %.

It is, however, preferred that the inner coating layer is in direct contact with the outer coating layer.

In an embodiment, in the coating according to the invention

- the amount of the inner polymerisable compound in the inner coating composition is 50-99 %, the amount of the antimicrobial agent in the inner coating composition is 0.05-15 %, and the amount of the initiator in the inner coating composition is 0.01-6 %, all based on the total dry weight of the inner coating composition; and
- the amount of the inorganic particles with a polymerisable group and a hydrophilic polymer chain attached to one or more of the inorganic

particles in the outer coating composition is 40-95 %, the amount of the cell-binding moiety in the outer coating composition is 1-50 %, and the amount of the initiator in the outer coating composition is 0.01-6 %, all based on the total dry weight of the outer coating composition.

The invention further relates to an article comprising a coating according to the invention.

Suitably examples of such articles include implants, including those replacing or augmenting cartilage, bone implants or replacements, bone fixation devices, maxillofacial implants, orthopaedic cements and glues comprised of polymers, resins, metals, alloys, plastics and combinations thereof, nails, screws, plates, fixator devices, wires and pins and the like, stents, stent grafts, anastomotic connectors, synthetic patches, leads, electrodes, needles, guide wires, catheters, surgical meshes, sensors, surgical instruments, angioplasty balloons, wound drains, shunts, tubings, infusion sleeves, urethral inserts, pellets, blood oxygenators, pumps, vascular grafts, vascular access ports, heart valves, annuloplasty rings, sutures, surgical clips, surgical staples, pacemakers, implantable defibrillators, neurostimulators, cerebrospinal fluid shunts, implantable drug pumps, drug delivery devices, spinal cages, artificial discs, replacement devices for nucleus pulposus, ear tubes, intraocular lenses, tubing used in minimally invasive surgery, membranes, diagnostic devices, biosensors such as for glucose or troponin, microtiter plates, microwell plates, microarrays (such as lab-on-a-chip), immunoassays, microfluidic devices, cell binding applications, Enzyme-Linked Immuno Sorbent Assay (ELISA), diagnostic test strips, blood collection tubes and capillaries, and diabetes device management devices.

Furthermore, the invention relates to a method of forming a coating comprising the steps of

a) applying an inner coating composition on a substrate, the inner coating composition comprising
   i) an inner polymerisable compound comprising a polymerisable group,
   ii) an antimicrobial agent, and
   iii) an initiator, and
b) curing the inner coating composition by activating the initiator, thereby forming an inner coating layer,
c) applying an outer coating composition on the inner coating layer, the outer coating composition comprising:
   i) inorganic particles with a polymerisable group and a hydrophilic polymer chain attached to one or more of the inorganic particles,
   ii) a cell-binding moiety,
   iii) an initiator, and
   iv) optionally, an outer polymerisable compound comprising a polymerisable group,
d) curing the outer coating composition, thereby forming an outer coating layer.

The inner coating composition may be applied onto the substrate by any process known in the art of wet coating deposition in one or multiple steps. Examples of suitable processes are spin coating, dip coating, spray coating, flow coating, meniscus coating, capillary coating and roll coating. A substrate may be totally coated or partially coated with the inner coating composition. Also partial cross-linking of the inner coating composition and removal of the non cross-linked part is possible, by for instance but not limited to photolithography.

After application and curing of the inner coating composition, the outer coating composition is applied on the inner coating layer and subsequently cured. Application and curing of the outer coating composition can be performed using similar processes as used for applying and curing

the inner coating composition. Optional further processing steps such as a heat treatment or radiation treatment are possible.

Various substrates may be used as a substrate in the method of the invention. Suitable substrates are for example flat or curved, rigid or flexible substrates including films of for example polycarbonate, polyester, polyvinyl acetate, polyurethanes, polyaryletherketones (PAEKs), such as polyether ether ketone (PEEK), polyvinyl pyrollidone, polyvinyl chloride, polyimide, polyethylene naphthalate, polytetrafluoro ethylene, nylon, polynorbornene, polyolefins, polyamides, polystryrene or amorphous solids, for example glass, or crystalline materials, such as silicon or gallium arsenide. Metallic substrates such as steel, titanium, or other metal alloys may also be used. Inorganic substrates such as TiO_2 may also be used.

A free-standing coating obtainable by a process according to the invention may be obtained by preparing a film or coating on a substrate and subsequently removing the film or coating from the substrate after polymerisation.

Examples

Synthesis of PEGDAA (polyethylene glycol diacrylamide)

20 g (13.3 mmol) of PEG-diamine (M_n 1500 g/mol; Aldrich) was azeotropically distilled in 400 ml of toluene under nitrogen, removing about 100 ml of toluene. The solution was cooled at room temperature under nitrogen and then cooled in an ice bath. 50 ml of dichloromethane (Merck) was added. 4.04 g (39.7 mmol) of triethylamine was added dropwise followed by the dropwise addition of 3.48 g (39.7 mmol) of acryloyl chloride (used without further purification). The reaction proceeded overnight under nitrogen. The solution was cooled in an ice bath to precipitate $NEt_3 \cdot HCl$ salts and was then filtrated. After adding 1 % (w/w) Irganox 1035, the filtrate was concentrated under vacuum. The concentrate was redissolved in

75 ml of dichloromethane, followed by precipitation in 1.5 l ice cold diethyl ether. The product was collected by filtration and subsequent washing with diethyl ether.

^{1}H-NMR ($CDCl_3$, 22 °C.) δ (TMS) = 6.7 ppm (2H, −N*H*−); 6.2 & 6.1 ppm (4H, C*H*$_2$=CH−); 5.6 ppm (2H, CH_2=C*H*−); 3.6 ppm (164H, −O−C*H*$_2$−CH_2− and −O−C*H*$_2$−C*H*$_2$−CH_2−NH−); 1.8 ppm (4H, −O−CH_2−CH_2−C*H*$_2$−NH−).

The NMR spectrum confirmed the formation of PEGDAA. Comparing the integration of the NMR peaks at 6.2 and 6.1 ppm to the peak at 1.8 ppm, about 99 % of the PEG-diamine was estimated to be converted into PEGDAA. The IR spectrum confirmed the formation of PEGDAA.

Synthesis of the GRGDS Moiety

A GRGDS (Gly-Arg-Gly-Asp-Ser) moiety based on polyethylene glycol (PEG) was synthesised. The synthesis was done by introducing cross-linkable acrylamide groups to an α-amino-ω-carboxy PEG by conversion of the amino end-group of PEG with two methylene bisacrylamide (MBA) molecules and subsequently converting the carboxylic acid end group of PEG with protected GRGDS via an active ester intermediate (Fig. 1). The last step is the deprotection of the GRGDS group with trifluoroacetic acid (TFA).

509 mg of α-amino-ω-carboxy PEG hydrochloride with a molecular weight of 3000 (1 eq, 0.157 mmol) and 123 mg methylene bisacrylamide (5.0 eq, 0.80 mmol) were dissolved in 2.5 ml methanol in a nitrogen atmosphere. 42 mg of triethylamine (TEA) (2.6 eq, 0.42 mmol) was added and the reaction mixture was stirred at 50 °C for 24 h. Then, the mixture was diluted with 0.5 ml methanol and precipitated in diethylether (350 ml) twice. The resulting white precipitate was dried *in vacuo* to yield Intermediate 1 (400 mg, 63 %).

594 mg of Intermediate 1 (1 eq, 0.165 mmol), 28 mg (1.2 eq, 0.401 mmol) 1-hydroxy-7-azabenzotriazole (HOAT) and 37 mg (1.2 eq, 0.193 mmol) 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) were dissolved in 12 ml of dimethylformamide (DMF) under nitrogen atmosphere. 116 µl (4 eq, 0.658 mmol) diisopropylethylamine (DIPEA) was added and the mixture was cooled on ice. After 15 min, 171 mg (1.1 eq, 0.175 mmol) protected GRGDS in 6 ml DMF was added. After 48 to 60 h, the solution was diluted with approximately 150 ml of dichloromethane (DCM). It is washed twice with 0.1 M HCl solution (150 ml) and the organic phase was dried over sodium sulfate to yield Intermediate 2. Conversion was confirmed by reducing the solvent *in vacuo*, and precipitating from approximately 300 ml of diethylether.

For deprotection of the GRGDS, the unprecipitated Intermediate 2 was redissolved in 8.5 ml of dry DCM and 8 ml trifluoracetic acid under nitrogen atmosphere. 87 µl of triisopropylsilane was added and the mixture was stirred for 3 h. Then, the solvent was reduced *in vacuo* and precipitated from 400 ml ice-cooled diethylether. The resulting off-white precipitate was dried *in vacuo* to yield the GRGDS moiety (443 mg, 65 %).

The functionalisation degree of the GRGDS moiety relative to the acrylamide groups of the GRDGS moiety was calculated from NMR data and purity of the compound was checked by LC-UV. NMR showed RGD:acrylamide ratios close to 1:1, indicating that not all of amino groups reacted twice with MBA in the first stage of the synthesis. The GRGDS moiety therefore consists of a mixture of molecules with no, one or two acrylamide groups. From LC-UV, it could be concluded that the product was 70 % pure. The main impurity comes from Intermediate 1 that was not coupled to GRGDS.

Preparation of coatings

The inner coating compositions were prepared by adding together all the components other than the antimicrobial agent (chlorhexidine, hereinafter "CHX", obtained from Aldrich). The CHX wad added last to form the inner coating composition. Irgacure® 819 photoinitiator (hereinafter, "Irg 819") was obtained from Aldrich.

GRGDS moiety, if any, was added to a commercially available VitroStealth® 43501 formulation comprising cross-linkable, PEG-modified SiO_2 nanoparticles, methylene bisacrylamide (hereinafter "MBA"), Irgacure® 184 photoinitiator (hereinafter, "Irg 184"), and a solvent of MeOH and *n*-propanol. More information about cross-linkable PEG-modified SiO_2 nanoparticles can be found in US Patents 8557897 and 8772373, which are each incorporated by reference in their entirety as if fully set forth herein.

The coatings were formed after subjecting the coating compositions to one day of stirring. First, the inner coating composition was applied at a constant application speed (automated application system) using a 40 μm roll-bar and cured by UV light (Fusion UV systems, D-bulb, N_2, 18 m/min, 1 J/cm^2 per run, 5 runs). Then the outer coating composition was applied on top (12 μm rollbar) and cured by UV light.

Inner coating compositions are shown in Table 1 and outer coating compositions are shown in Table 2, below. Solids components are listed as wt.% of the dry coating, whereas the total amount of solid components and the amount of solvent are listed as a wt.% based on the total weight of the inner or outer coating composition.

Table 1: Inner Coating Compositions

Num.	Total Solids	Solid Components				Solvent Component
		PEGDAA	Irg 819	Acrylic acid	CHX	MeOH
I-1	14	74	2	14	10	86
I-2	13	82	3	15	0	87
I-3	14	78	3	8	11	86
I-4	14	85	3	6.5	5.5	86

Table 2: Outer Coating Compositions

Num.	Total Solids	Solid Components				Solvent Components	
		SiO_2 NP	MBA	Irg 184	GRGDS Moiety	MeOH	*n*-propanol
O-1	8	94.5	5	0.5		46	46
O-2	7	90	4.5	0.5	5	46.5	46.5
O-3	7	85.5	4.5	0.5	9.5	46.5	46.5
O-4	7	76.5	4	0.5	19	46.5	46.5

For all examples except Example 2, coating compositions I-1, I-2, I-3, and I-4 were cured with a total dose of 5 J/cm^2, coating compositions O-1, O-2, and O-3 were cured with a total dose of 2 J/cm^2, and coating composition O-4 was cured with a total dose of 3 J/cm^2.

Example 1: Release of CHX

Coated PET sheets of 1 × 2 cm were formed by applying and then curing an inner coating composition to form an inner coating layer. In Experiments 1-2 and 1-3, an outer coating layer was formed by applying and then curing the outer coating composition on top of the inner coating layer. The coatings used in this experiment are as shown in Table 3:

Table 3: Coatings formed on PET sheets used in Example 1

Ex.	Inner Coating Formulation	Outer Coating Formulation
1-1	I-1	None
1-2	I-2	O-1
1-3	I-3	O-2

The coated PET sheets were placed in a vial containing 4 ml of PBS buffer (pH = 7, without Ca^{2+} or Mg^{2+}, Dubelco), so that the sheet was completely submerged. The vials were kept shaking at 100 rpm and 37 °C. The medium was exchanged for fresh PBS buffer at certain time intervals. CHX concentrations in the washing solutions were determined by UV-Vis spectroscopy. The absorption values at $\lambda = 255$ nm were converted to mass concentrations of chlorhexidine via a calibration curve and plotted against time in a cumulative way. Absorption values below 0.005 (*i.e.* CHX concentrations below 0.035 mg/l) were considered as noise and when observing those values the release experiment was stopped.

A slight reduction in total release is observed in Experiments 1-2 and 1-3 (2 layer systems) relative to Experiment 1-1 (a single layer system). As depicted in Fig. 2, the total release is only slightly lowered in Experiment 1-2 relative to Experiment 1-1, indicating that the outer coating layer quite permeable to CHX. When 5 % of GRGDS is mixed into the outer coating composition in Experiment 1-3, there is a greater reduction of total release. In general, CHX release rate can be controlled by the amount of added acrylic acid, the concentration of CHX in the coating, and the inner coating layer thickness.

Example 2: BSA adhesion

Coating samples were prepared in blood collection tubes of 4.5 cm length. The tubes were cleaned with a 1:1 vol.% mixture of *n*-propanol/methanol in an ultrasonic bath for 30 min and dried. Coatings

were applied by filling the tubes with formulation and emptying them with a plastic pipette. The coated tubes were left to dry upside down under ambient conditions for 30 min and subsequently UV cured with a D bulb (UV dose: 6 J/cm^2).

1.0 ml of a diluted ^{125}I-BSA solution was transferred into all coated and cured blood collection tubes. The filled BCT tubes were incubated overnight, then the ^{125}I-BSA solution was removed and the samples were washed three times with a PBS buffer solution. The BCT samples were then submerged in 20 ml LSC cocktail. The radioactive BSA that remains on the vials after washing were then measured relative to reference uncoated vials. All samples were measured at least in fivefold.

As depicted in Fig. 3, the normalised initial reduction of BSA adsorption (before CHX release) was similarly improved for any coating that included VitroStealth®.

As depicted in Fig. 4, a small reduction is seen in BSA adsorption reduction after CHX release.

As depicted in Fig. 5, virtually no deterioration of protein repellency was observed when the amount of GRGDS moiety was varied from 5 wt.% to 19 wt.% in the outer layer formulation. Thus, excellent non-biofouling properties of GRGDS moiety containing coatings could be maintained.

Example 3: Biocompatibility and cytotoxicity

A leaching study was performed, investigating if and how much uncured acrylic acid is leaching out of the coatings. Using the LC-UV technique, no acrylic acid could be detected (the detection limit was 5 ppm) for a 4 cm^2 coating area of 2 μm thickness in 2 ml of buffer. Thus, it is unlikely that acrylic acid leaching from such coating systems will pose a problem during *in vivo* applications.

Additionally, two layer coatings were also submitted for *in vitro* cytotoxicity and hemolysis tests. For determination of cytotoxicity, the viability cell culture to a 48 h exposure to extracts of the coating was determined. Coatings without CHX showed no cytotoxic effects and good hemocompatibility. Only in the case of CHX loaded coatings, a reduction in cell viability and hemocompatibility was observed, which indicates that the released CHX causes the adverse effects observed.

Example 4: Microbiological Evaluation

Samples were formed by forming coatings onto PET sheets using the procedure of Example 1. A bacterial suspension with a concentration of 2×10^4 bacteria/ml (*S. aureus* ATCC 49230) was prepared to yield a bacterial challenge concentrations in 50 µl droplets being 10^3 bacteria. First, one droplet of 50 µl bacterial suspension was placed in the middle of each coated or uncoated PET sheet and placed between two sheets of a Petrifilm. The Petrifilm was closed and incubated for 48 h. After the incubation period, the number of CGUs are read.

The results are shown below in Table 4. In the Table, "t.n.t.c." represents too numerous to count. The amount of CHX is shown in wt.% based on the total dry weight of the inner coating composition. The amount of GRGDS moiety is shown in wt.% based on the total dry weight of the outer coating composition. The amount of CHX and GRGDS moiety are mentioned below for convenience; no additional CHX or GRGDS moiety was added beyond that shown in Table 1 and Table 2, respectively.

Table 4: Example 4 Results

Sample	coating	CHX	GRGDS Moiety	Trial 1	Trial 2	Trial 3	average
				#CFU	#CFU	#CFU	#CFU
4-1	None	0	0	t.n.t.c	t.n.t.c	t.n.t.c	t.n.t.c
4-2	I-2/O-1	0	0	t.n.t.c	t.n.t.c	t.n.t.c	t.n.t.c
4-3	I-2/O-2	0	5	t.n.t.c	t.n.t.c	t.n.t.c	t.n.t.c
4-4	I-2/O-3	0	9.5	t.n.t.c	t.n.t.c	t.n.t.c	t.n.t.c
4-5	I-4/O-1	5.5	0	0	0	0	0
4-6	I-4/O-2	5.5	5	0	0	0	0
4-7	I-4/O-3	5.5	9.5	1	0	0	0.3
4-8	I-4/O-4	5.5	19	1	0	0	0.3

It is evident that all CHX containing samples show a near 100 % bacterial growth inhibition.

Example 5: Cell spreading

U2OS osteosarcoma cells were cultured in Dulbecco's modified Eagles Medium (DMEM)-low glucose supplemented with 10 % fetal calf serum (FBS) and 0.2 mM of ascorbic acid-2-phosphate (AA2P) denoted as DMEM/LG complete. U2OS cells were maintained at 37 °C in a humidified 5 % CO_2 atmosphere, and passaged at 80-90 % confluency using trypsin/EDTA. Samples were sterilised using 70 % ethanol and sterile demineralised water and placed in a sterile 6 well suspension culture plate (Cellstar, Greiner Bio-one).

Cells with a concentration of $5 \times 10^3/cm^2$ suspended in DMEM/LG complete were allowed to adhere to the sample surface (surface of one well is 11.5 cm^2) for 24 hours at 37 °C in a humidified 5 % CO_2 atmosphere. After 24 hours images were taken of the U2OS cell spreading on the sample surface.

A first set of samples were tested shortly after placing the sample in PBS. A second set of samples were placed in sterile demineralised water

for 48 hours at 37 °C in order to release the CHX. The demineralised water was refreshed 8 times during the 48 hours.

The results are shown in Table 5 below. Cell growth is reported using a scale of 0-5, with 0 being no cell growth and 5 being the highest amount of cell growth observed in this experiment. The amount of CHX is shown in wt.% based on the total dry weight of the inner coating composition. The amount of GRGDS moiety is shown in wt.% based on the total dry weight of the outer coating composition. The amount of CHX and GRGDS moiety are mentioned below for convenience; no additional CHX or GRGDS moiety was added beyond that shown in Table 1 and Table 2, respectively.

Table 5: Example 5 Results

Sample	Coating	CHX	GRGDS Moiety	First Set		Second Set (48 h leakage)
				Cell Growth Trial 1	Cell Growth Trial 2	Cell growth
5-1	None	0	0	5	Not tested	Not tested
5-2	I-2/O-1	0	0	3	3	2
5-3	I-2/O-2	0	5	3	3	3
5-4	I-2/O-3	0	9.5	4	3	4
5-5	I-4/O-1	5.5	0	1	1	1
5-6	I-4/O-2	5.5	5	1	2	3
5-7	I-4/O-3	5.5	9.5	4	2	4
5-8	I-4/O-4	5.5	19	4	3	3

From the results presented in Table 5 it is clear that the samples with CHX and without GRGDS moiety only scarcely admit cell growth. At 9.5-19 wt.% GRGDS moiety the negative effect of CHX seems to be compensated for.

48 h in demineralised water, which depleted the CHX reservoir, increases cell spreading and survival as expected.

Fig. 6 is a microscope image of U2OS cell spreading from the first set of experiments on coatings with 5.5 wt.% CHX and increasing concentrations of GRGDS moiety.

Example Conclusions

Antifouling properties of the tested two layer coating systems remained very high during and after CHX release. Antifouling properties and release of CHX are not significantly influenced by the presence of the GRGDS moiety. In particular the tested two layer coatings are promising with respect to double (antifouling/biocidal) and triple action (antifouling/biocidal/cell adhesive). An anti-bacterial effect was demonstrated at 5 wt.% CHX. Relative to uncoated samples, cell spreading/growth is decreased for two-layer coatings that do not contain a GRGDS moiety and the presence of CHX reduces cell spreading/growth even further. However, 19 wt.% GRGDS moiety was shown to reduce the negative effect of CHX on cell spreading.

Claims

1. A coating, comprising

a) an inner coating layer formed from an inner coating composition, the inner coating composition comprising,

i) an inner polymerisable compound comprising a polymerisable group,

ii) an antimicrobial agent, and

iii) an initiator, and

b) an outer coating layer formed from an outer coating composition, the outer coating composition comprising

i) inorganic particles with a polymerisable group and a hydrophilic polymer chain attached to one or more of the inorganic particles,

ii) a cell-binding moiety,

iii) an initiator, and

iv) optionally, an outer polymerisable compound comprising a polymerisable group.

2. The coating according to claim 1, wherein the antimicrobial agent forms a complex with a complexing moiety and the complexing moiety is covalently bound to the inner polymerisable compound, or wherein the antimicrobial agent is entrapped in the inner coating layer.

3. The coating according to claim 1 or 2, wherein the inner polymerisable compound comprises acrylic acid.

4. The coating according to any one of claims 1-3, wherein the polymerisable group of the inner polymerisable compound comprises a (meth)acrylate or (meth)acrylamide group.

5. The coating according to any one of claims 1-4, wherein the inner polymerisable compound comprises a hydrophilic polymer

6. The coating according to any one of claims 1-5, wherein the inner polymerisable compound comprises poly(alkylene oxide) or polyvinylpyrrolidone.

7. The coating according to any one of claims 1-6, wherein the antimicrobial agent comprises one or more selected from the group consisting of silver nanoparticles, ionic silver, and/or a biguanide.

8. The coating according to any one of claims 1-7, wherein the inorganic particles comprise one or more selected from the group consisting of SiO_2, TiO_2, ZnO_2, SnO_2, Am-SnO_2, ZrO_2, Sb-SnO_2, Al_2O_3, Au and Ag.

9. The coating according to any one of claims 1-8, wherein the hydrophilic polymer chain comprises poly(alkylene oxide).

10. The coating according to any one of claims 1-9, wherein the cell-binding moiety further comprises a polymerisable group.

11. The coating according to any one of claims 1-10, wherein the cell-binding moiety further comprises a hydrophilic polymer chain.

12. The coating according to any one of claims 1-11, wherein the cell-binding moiety comprises one or more selected from the group consisting of a Arg-Gly-Asp (RGD)-containing peptide sequence, a Tyr-Ile-Gly-Ser-Arg (YIGSR)-containing peptide sequence, and a Ile-Lys-Val-Ala-Val (IKVAV)-containing peptide sequence.

13. The coating according to any one of claims 1-12, wherein the cell-binding moiety comprises a Arg-Gly-Asp (RGD)-containing peptide sequence

14. The coating according to any one of claims 1-13, wherein the cell-binding moiety comprises a Gly-Arg-Gly-Asp-Ser (GRGDS) peptide.

15. The coating according to any one of claims 1-14, wherein

- the amount of the inner polymerisable compound in the inner coating composition is 50-99 %, the amount of the antimicrobial agent in the inner coating composition is 0.05-15 %, and the amount of the initiator in the inner coating composition is 0.01-6 %, all based on the total dry weight of the inner coating composition; and
- the amount of the inorganic particles with a polymerisable group and a hydrophilic polymer chain attached to one or more of the inorganic particles in the outer coating composition is 40-95 %, the amount of the cell-binding moiety in the outer coating composition is 1-50 %, and the amount of the initiator in the outer coating composition is 0.01-6 %, all based on the total dry weight of the outer coating composition.

16. The coating according to any one of claims 1-15, wherein the inner coating is in direct contact with the outer coating.

17. An article comprising a coating according to any one of claims 1-16.

18. A method of forming a coating comprising the steps of

a) applying an inner coating composition on a substrate, the inner coating composition comprising

i) an inner polymerisable compound comprising a polymerisable group,

ii) an antimicrobial agent, and

iii) an initiator, and

b) curing the inner coating composition by activating the initiator, thereby forming an inner coating layer,

c) applying an outer coating composition on the inner coating layer, the outer coating composition comprising:

i) inorganic particles with a polymerisable group and a hydrophilic polymer chain attached to one or more of the inorganic particles,

ii) a cell-binding moiety,

iii) an initiator, and

iv) optionally, an outer polymerisable compound comprising a polymerisable group,

d) curing the outer coating composition, thereby forming an outer coating layer.

Fig. 1

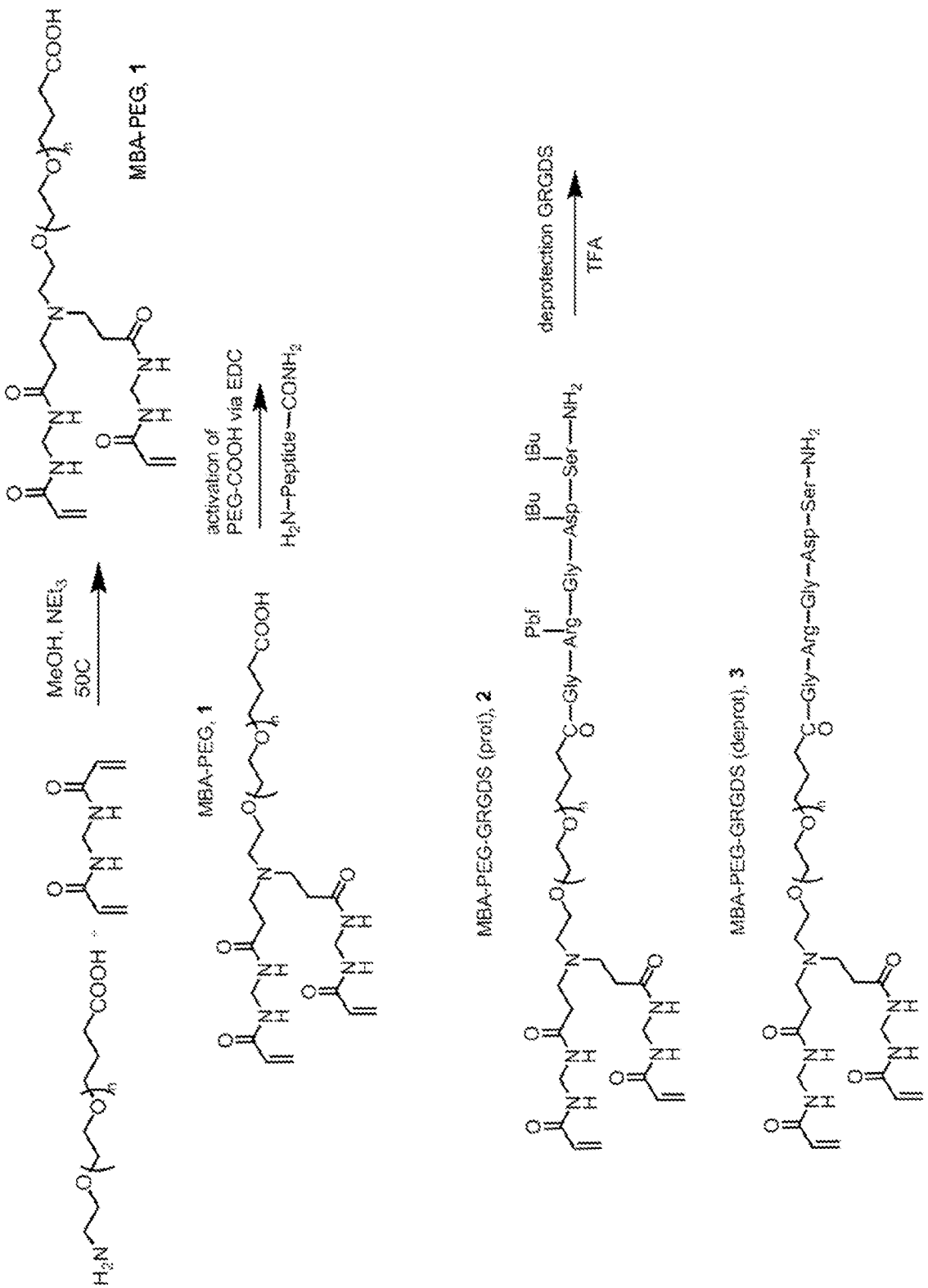

MeOH, NEt3
50C
MBA-PEG, 1
activation of
PEG-COOH via EDC
H2N–Peptide–CONH2
MBA-PEG, 1
MBA-PEG-GRGDS (prot), 2
deprotection GRGDS
TFA
MBA-PEG-GRGDS (deprot), 3

Fig. 2

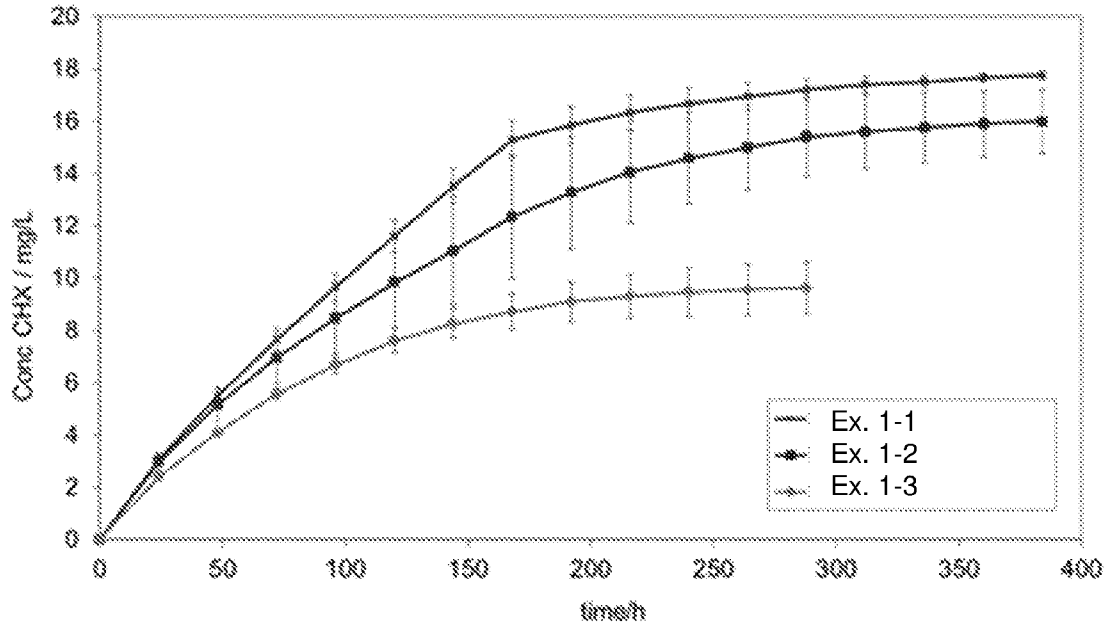

Conc CHX / mg/L
0
2
4
6
8
10
12
14
16
18
20
0
50
100
150
200
250
300
350
400
time/h
Ex. 1-1
Ex. 1-2
Ex. 1-3

Fig. 3

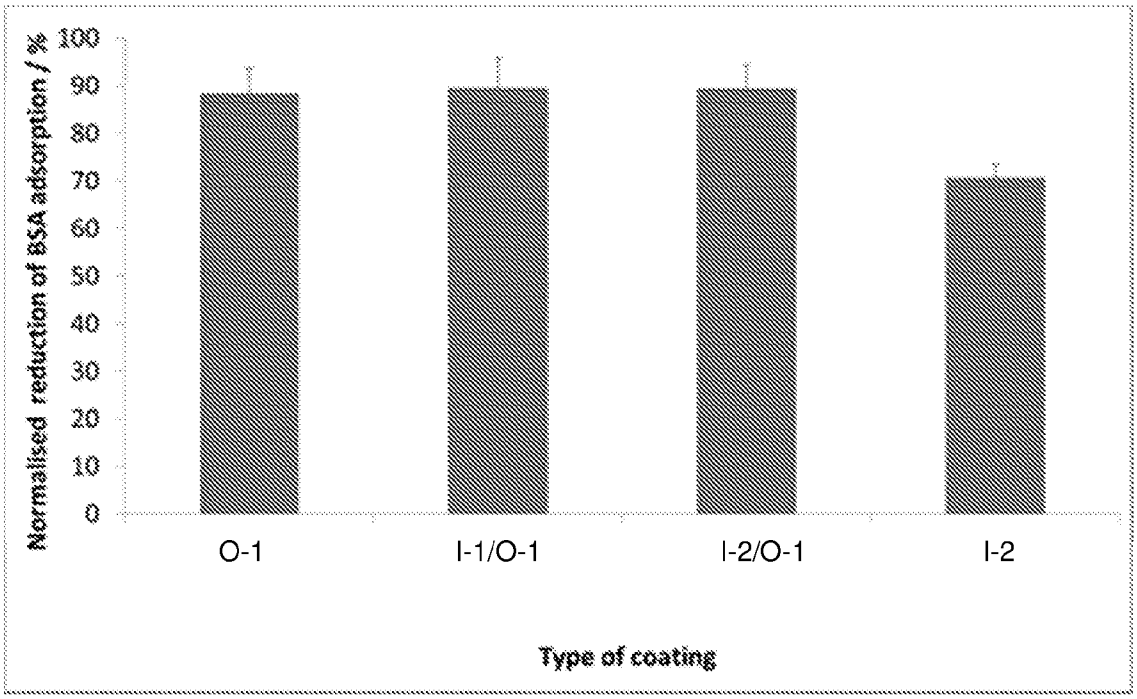

Normalised reduction of BSA adsorption / %
0
10
20
30
40
50
60
70
80
90
100
O-1
I-1/O-1
I-2/O-1
I-2
Type of coating

Fig. 4

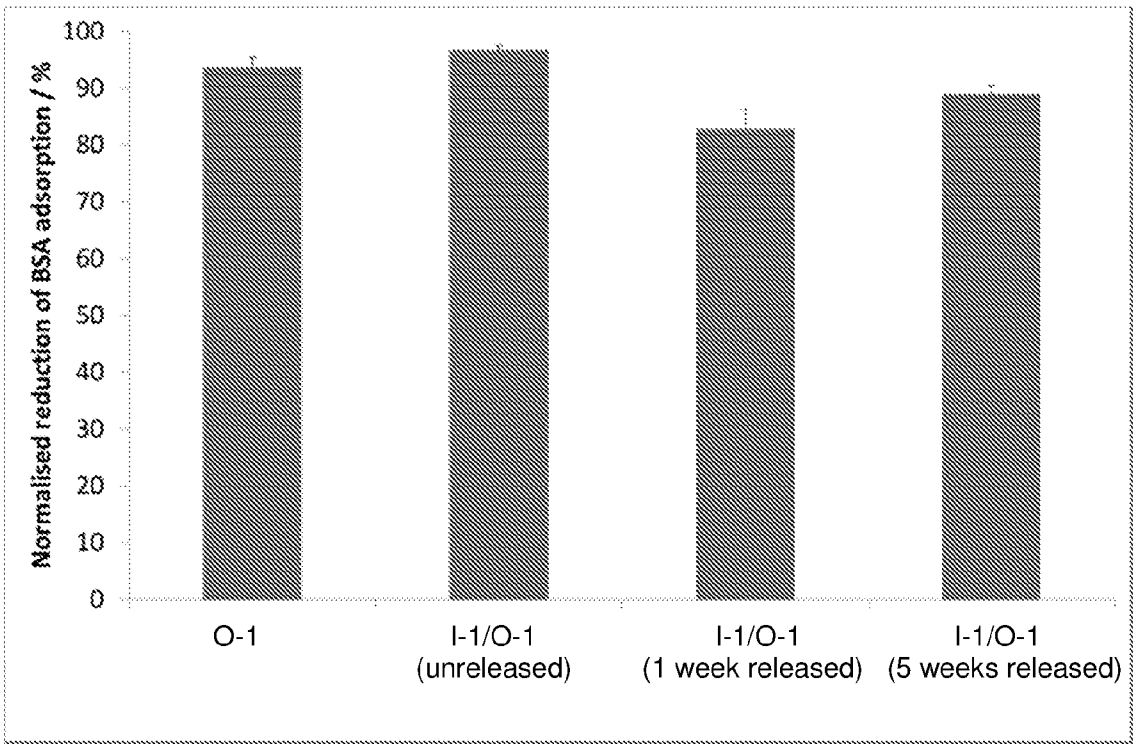

Normalised reduction of BSA adsorption / %
100
90
80
70
60
50
40
30
20
10
0
O-1
I-1/O-1 (unreleased)
I-1/O-1 (1 week released)
I-1/O-1 (5 weeks released)

Fig. 5

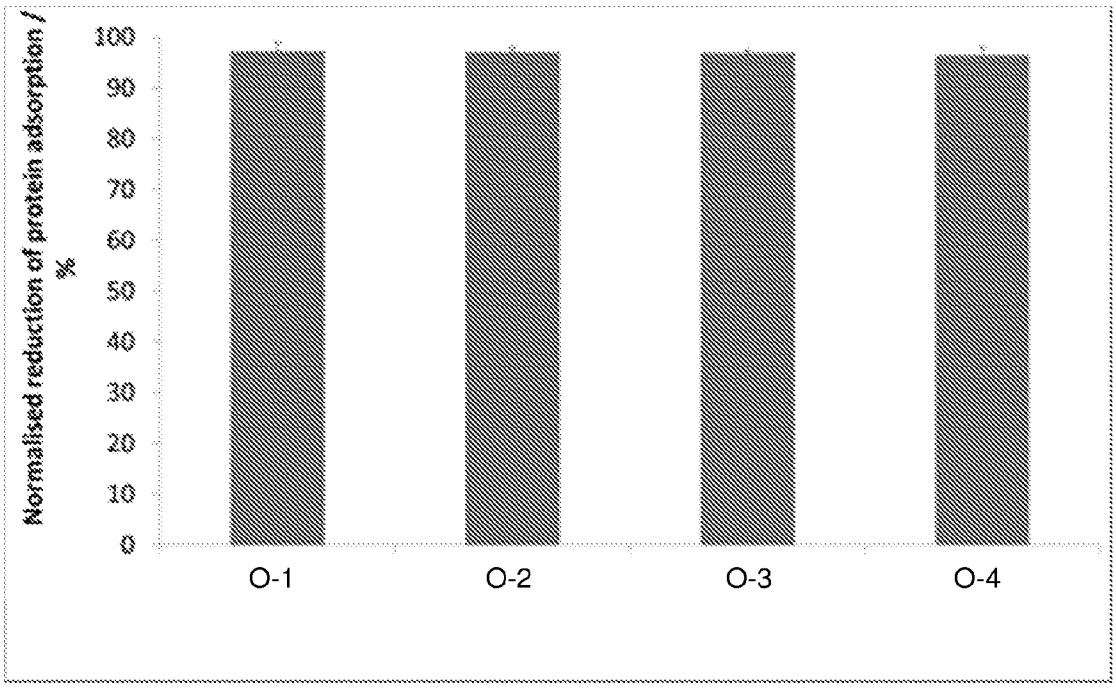
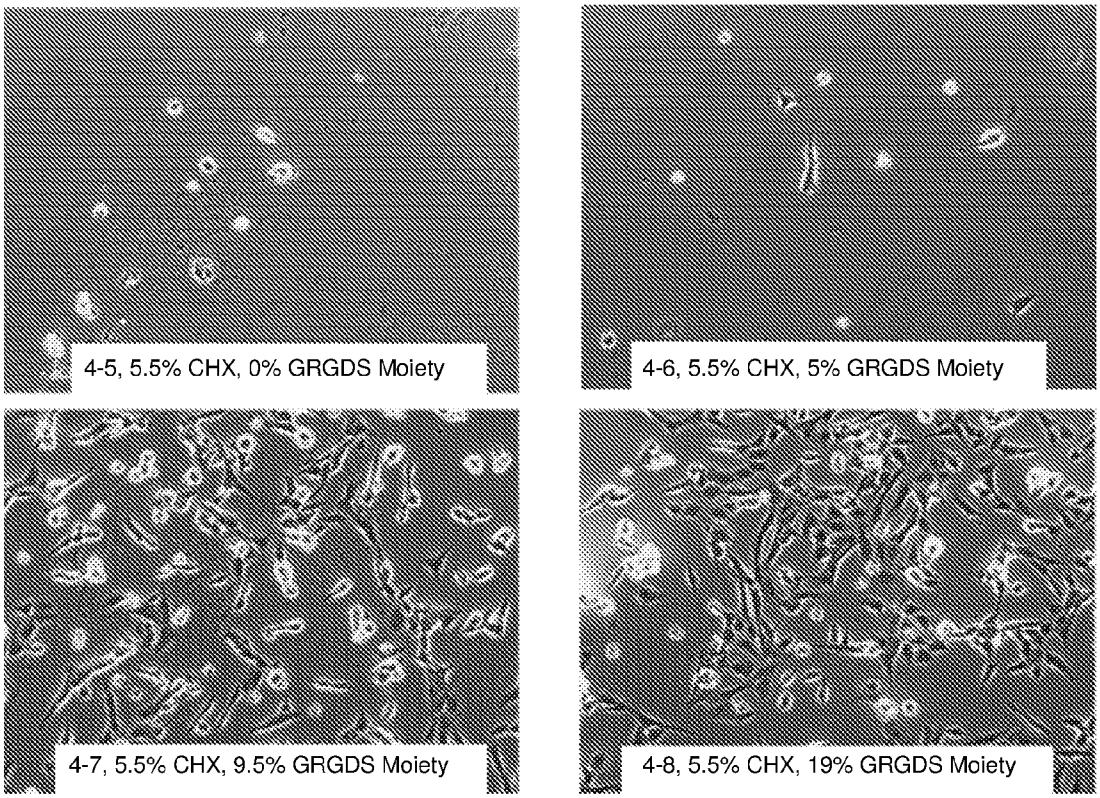

Normalised reduction of protein adsorption / %
100
90
80
70
60
50
40
30
20
10
0
O-1
O-2
O-3
O-4

Fig. 6


4-5, 5.5% CHX, 0% GRGDS Moiety
4-6, 5.5% CHX, 5% GRGDS Moiety
4-7, 5.5% CHX, 9.5% GRGDS Moiety
4-8, 5.5% CHX, 19% GRGDS Moiety

INTERNATIONAL SEARCH REPORT

International application No
PCT/NL2016/050159

A. CLASSIFICATION OF SUBJECT MATTER
INV. C09D5/14 C09D5/16 C09D4/06 C09D1/00 C09D7/12
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)
C09D

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, CHEM ABS Data, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2014/228466 A1 (LIN JANICE [US] ET AL) 14 August 2014 (2014-08-14) claims 1,3-4,9-15,17-18	1,3,4,7, 10-14,18
A	WO 2014/070792 A1 (ARISTE MEDICAL INC [US]) 8 May 2014 (2014-05-08) claims 39, 41-43,45-47	1,4

☐ 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

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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	Date of mailing of the international search report
11 July 2016	29/07/2016

Name and mailing address of the ISA/	Authorized officer
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/NL2016/050159

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2014228466 A1	14-08-2014	AU 2014216565 A1	13-08-2015
		CA 2900355 A1	21-08-2014
		EP 2956510 A1	23-12-2015
		JP 2016511787 A	21-04-2016
		US 2014228466 A1	14-08-2014
		WO 2014126862 A1	21-08-2014
WO 2014070792 A1	08-05-2014	AU 2013338051 A1	11-06-2015
		CA 2889981 A1	08-05-2014
		CN 104884545 A	02-09-2015
		EP 2912121 A1	02-09-2015
		US 2015275026 A1	01-10-2015
		WO 2014070792 A1	08-05-2014