Claims

1. A composite superhydrophobic material suitable for use in surface-enhanced Raman scattering, the material comprising:

a substrate layer comprising a set of positively or negatively charged functional groups;

a metal nanoparticle coating on top of the substrate layer, comprising a set of negatively or positively charged functional groups;

a metal layer on top of the metal nanoparticle coating; and

a first superhydrophobic layer on top of the metal layer, wherein:

the metal nanoparticle coating comprises a first set and a second set of metal nanoparticles, where the first set of metal nanoparticles has a diameter that is from 0.1 to 90**%** smaller than the diameter of the second set;

the metal of the first set of metal nanoparticles is gold and/or silver;

the metal of the second set of metal nanoparticles is gold and/or silver; and

the charged functional groups on the substrate layer and metal nanoparticle coating are electronically complementary to one another, wherein

the first set of metal nanoparticles are nanocubes and the second set of metal nanoparticles are nanopolyhedrons having more than six faces and are selected from one or more nanopolyhedra having from 7 to 30 faces.

2. The composite superhydrophobic material according to Claim 1, wherein:

the density of the metal nanoparticle coating on the surface of the substrate layer is from 2 to 20 particles/μm^2, such as from 2.5 to 10 particles/μm^2, such as from 3 to 5 particles/μm^2, such as 4.4 particles/μm^2; and/or

the composite material further comprises a second superhydrophobic layer sandwiched between the metal nanoparticle coating and the metal layer.

3. The composite superhydrophobic material according to Claim 1 or Claim 2, wherein the material displays a static contact angle with water of greater than 150°, such as from 151° to 170°, such as 155° to 165°.

4. The composite superhydrophobic material according to any one of the preceding claims, wherein:

the first set of nanoparticles have an average diameter of from 10 to 250 nm, such as from 20 to 100 nm, such as from 30 to 50 nm, such as from 130 to 240 nm, such as from 135 to 230 nm; and/or

the second set of nanoparticles have an average diameter of from 250 to 1,000 nm, such as from 300 to 600 nm, such as from 311 to 540 nm.

5. The composite superhydrophobic material according to any one of the preceding claims, wherein:

(ia) the first set of metal nanoparticles has a diameter that is from 0.2 to 60% smaller than the diameter of the second set, such as from 0.5 to 57%, such as from 1 to 55%, such as from 10 to 50%, such as from 25 to 45% smaller than the diameter of the second set, such as from 61 to 89% smaller than the diameter of the second set, such as from 75 to 85%, such as from 79 to 83%; and/or

(iia) the composite has a root mean square surface roughness of from 30 to 150 nm, such as 40 to 140 nm, such as from 50 to 125 nm, such as 110 to 125 nm; and/or

(iiia) the total number of particles in the second set of nanoparticles to the total number of particles in the first set of nanoparticles on the composite material is from 1:10 to 10:1, such as from 1:5 to 5:1, such as from 2:1 to 4:1.

6. The composite superhydrophobic material according to any one of the preceding claims, wherein the set of charged functional groups on the substrate layer are positively charged and the charged functional groups on the metal nanoparticle coating are negatively charged, optionally wherein:

the set of positively charged functional groups on the substrate layer are ammonium ions; and/or

the set of negatively charged functional groups on the first and second set of metal nanoparticles are carboxylate ions or alkoxide ions.

~~7. The composite superhydrophobic material according to Claim 6, wherein:~~

~~the set of positively charged functional groups on the substrate layer are ammonium ions; and/or~~

~~the set of negatively charged functional groups on the first and second set of metal nanoparticles are carboxylate ions or alkoxide ions.~~

~~8~~7. The composite superhydrophobic material according to any one of Claims 1 to 5, wherein the set of charged functional groups on the substrate layer are negatively charged and the charged functional groups on the metal nanoparticle coating are positively charged, optionally wherein:

the set of negatively charged functional groups on the substrate layer are carboxylate ions or alkoxide ions; and/or

the set of positively charged functional groups on the first and second set of metal nanoparticles are ammonium ions.

~~9. The composite superhydrophobic material according to Claim 8, wherein:~~

~~the set of negatively charged functional groups on the substrate layer are carboxylate ions or alkoxide ions; and/or~~

~~the set of positively charged functional groups on the first and second set of metal nanoparticles are ammonium ions.~~

~~10~~8. The composite superhydrophobic material according to any one of the preceding claims, wherein:

(ib) the metal layer is from 5 to 53 nm thick, such as from 15 to 43 nm thick, such as from 20 to 33 nm thick, such as from 25 to 30 nm thick; and/or

(iib) the metal layer comprises a silver layer, a gold layer or a silver and gold layer, optionally wherein the silver layer, a gold layer or a silver and gold layer is from 5 to 48 nm thick, such as from 10 to 38 nm thick, such as from 15 to 28 nm thick, such as from 20 to 25 nm thick; and/or

(iiib) there is direct attachment between the metal layer and the substrate layer through gaps between nanoparticles in the metal nanoparticle coating; and/or

(ivb) the first superhydrophobic layer comprises a C_{10} to C_{20} thiol that is unsubstituted or substituted by one or more fluoro groups, optionally wherein the first superhydrophobic layer comprises one or more of the group consisting of 1-dodecanethiol, 1-hexadecanethiol, and *1H*,*1H*,2*H*,2*H*-perfluorodecanethiol; and/or

(vb) the second superhydrophobic layer comprises a C_{10} to C_{20} thiol that is unsubstituted or substituted by one or more fluoro groups, optionally wherein the second superhydrophobic layer comprises one or more of the group consisting of 1-dodecanethiol, 1-hexadecanethiol, and *1H*,*1H*,2*H*,2*H*-perfluorodecanethiol; and/or

(vib) the first and second set of nanoparticles are silver nanoparticles.

~~11. The composite superhydrophobic material according to any one of the preceding claims, wherein the first set of metal nanoparticles are nanocubes and the second set of metal nanoparticles are nanopolyhedrons having more than six faces and are selected from one or more nanopolyhedra having from 7 to 30 faces.~~

~~12~~9. The composite superhydrophobic material according to according to any one of the preceding claims~~Claim 11~~, wherein the second set of metal nanoparticles are nanooctahedra.

~~13. The composite superhydrophobic material according to any one of the preceding claims, wherein the first and second set of nanoparticles are silver nanoparticles.~~

~~14~~10. A kit of parts comprising:

(ai) a composite superhydrophobic material as described in any one of Claims 1 to ~~13~~9; and

(bi) a urine analysis formulation comprising silver and/or gold nanoparticles coated with a boronic acid comprising a thiol group, optionally wherein:
the nanoparticles are formed from the same metal(s) used in the composite superhydrophobic material; and/or
the boronic acid is 4-mercaptophenylboronic acid.

~~15~~11. A method of predicting increased risk of spontaneous miscarriage, the method comprising the steps of:

(a) providing an aqueous solution comprising the non-salt components of a urine sample obtained from a subject;

(b) reacting the aqueous solution with a urine analysis formulation comprising silver and/or gold nanoparticles coated with a boronic acid comprising a thiol group for a first period of time to provide a complexed sample, optionally wherein the nanoparticles are formed from the same metal(s) used in the composite superhydrophobic material and/or the boronic acid is 4-mercaptophenylboronic acid;

(c) placing the complexed sample onto a composite superhydrophobic material as described in any one of Claims 1 to ~~13~~ 9 and removing the water by evaporation to provide a dried sample; and

(d) subjecting the dried sample to Raman spectroscopy to determine the abundance of pregnane and tetrahydrocortisone and then predicting the risk of spontaneous miscarriage based on the formula:

$$\frac{Pregnane\ Abundance}{(Pregnane\ Abundance + \text{Tetrahydrocortisone Abundance})} x100\%$$

wherein a value lower than a threshold value is indicative of increased risk of spontaneous miscarriage, optionally wherein:
the first period of time is from 3 to 48 hours, such as 3 hours; and/or
the Raman spectroscopy is conducted using a laser excitation wavelength of 532 nm, using a laser power of from 0.01 to 1 mW and an acquisition time of from 1 to 60 seconds, optionally wherein the laser power is 0.2 mW and the acquisition time is 1 second.

~~16~~12. The method according to Claim ~~15~~11, wherein the aqueous solution provided in step (a) of the process is obtained by the following steps:

(i) adding a urine sample comprising salt and non-salt components obtained from a subject onto a reverse phase column to provide a loaded column;

(ii) removing the salt components from the loaded column by eluting with water, then removing and isolating the non-salt components from the loaded column by eluting with a C_{1-4} alcohol (e.g. methanol) and then removing the C_{1-4} alcohol; and

(iii) adding an aqueous solution to the isolated non-salt components to provide the aqueous solution of step (a).

~~17. The method according to Claim 15 or Claim 16, wherein the Raman spectroscopy is conducted using a laser excitation wavelength of 532 nm, using a laser power of from 0.01 to 1 mW and an acquisition time of from 1 to 60 seconds, optionally wherein the laser power is 0.2 mW and the acquisition time is 1 second.~~

~~18~~13. A method of making a composite superhydrophobic material suitable for use in surface-enhanced Raman scattering, the method comprising:

(ia) providing a metal-coated composite material comprising:

a substrate layer comprising a set of positively or negatively charged functional groups;

a metal nanoparticle coating on top of the substrate layer, comprising a set of negatively or positively charged functional groups, where the metal nanoparticle coating comprises a first set and a second set of metal nanoparticles, where the first set of metal nanoparticles has a diameter that is from 0.1 to 90**%** smaller than the diameter of the second set; and

a metal layer on top of the metal nanoparticle coating; and

(ib) immersing the metal-coated composite material in a solution comprising a superhydrophobic material to form a superhydrophobic layer on top of the metal layer, thereby forming the composite superhydrophobic material, wherein:

the metal of the first set of metal nanoparticles is gold and/or silver;

the metal of the second set of metal nanoparticles is gold and/or silver; and

the charged functional groups on the substrate layer and metal nanoparticle coating are electronically complementary to one another, wherein

the first set of metal nanoparticles are nanocubes and the second set of metal nanoparticles are nanopolyhedrons having more than six faces and are selected from one or more nanopolyhedra having from 7 to 30 faces.

~~19~~14. The method according to Claim ~~18~~13, wherein the metal-coated composite material is formed by:

(A) providing a metal nanoparticle coated substrate comprising a substrate layer that comprises a set of positively or negatively charged functional groups and a metal nanoparticle coating on top of the substrate layer that comprises a set of negatively or positively charged functional groups, where the metal nanoparticle coating comprises a first set and a second set of metal nanoparticles; and

(B) depositing a metal layer on top of the metal nanoparticle coated substrate, optionally wherein the deposition is by thin-film deposition by thermal evaporation of a metal, optionally wherein:

(id) the metal-coated composite material comprises a further superhydrophobic layer sandwiched between the metal nanoparticle coating and the metal layer; and/or

(iid) the metal layer is from 5 to 53 nm thick, such as from 15 to 43 nm thick, such as from 20 to 33 nm thick, such as from 25 to 30 nm thick; and/or

(iiid) the metal layer comprises a silver layer, a gold layer or a silver and gold layer, optionally wherein the silver layer, gold layer or silver and gold layer is from 5 to 48 nm thick, such as from 10 to 38 nm thick, such as from 15 to 28 nm thick, such as from 20 to 25 nm thick.

~~20. The method according to Claim 19, wherein:~~

~~(id) the metal-coated composite material comprises a further superhydrophobic layer sandwiched between the metal nanoparticle coating and the metal layer; and/or~~

~~(iid) the metal layer is from 5 to 53 nm thick, such as from 15 to 43 nm thick, such as from 20 to 33 nm thick, such as from 25 to 30 nm thick; and/or~~

~~(iiid) the metal layer comprises a silver layer, a gold layer or a silver and gold layer, optionally wherein the silver layer, gold layer or silver and gold layer is from 5 to 48 nm thick, such as from 10 to 38 nm thick, such as from 15 to 28 nm thick, such as from 20 to 25 nm thick.~~

~~21~~15. The method according to ~~any one of~~ Claim~~s~~ ~~18~~ 13 or Claim 14~~to 20~~, wherein the metal nanoparticle coated substrate is formed by contacting a substrate comprising a set of positively or negatively charged functional groups with a solution comprising metal nanoparticles comprising a set of negatively or positively charged functional groups, where the metal nanoparticles comprise a first and second set of metal nanoparticles.

~~22~~16. The method according to ~~any one of~~ Claim~~s~~ ~~19~~ 14 or Claim 15 ~~to 21~~, wherein:

(ie) the first set of nanoparticles have an average diameter of from 10 to 250 nm, such as from 20 to 100 nm, such as from 30 to 50 nm, such as from 130 to 240 nm, such as from 135 to 230 nm; and/or

(iie) the second set of nanoparticles have an average diameter of from 250 to 1,000 nm, such as from 300 to 600 nm, such as from 311 to 540 nm; and/or

(iiie) the first set of metal nanoparticles has a diameter that is from: 0.2 to 60% smaller than the diameter of the second set, such as from 0.5 to 57**%** such as from 1 to 55%, such as from 10 to 50%, such as from 25 to 45% smaller than the diameter of the second set, such as from 61 to 89% smaller than the diameter of the second set, such as from 75 to 85%, such as from 79 to 83% smaller than the diameter of the second set; and/or

(ive) the composite has a root mean square surface roughness of from 30 to 150 nm, such as 40 to 140 nm, such as from 50 to 125 nm, such as 110 to 125 nm; and/or

(ve) the total number of particles in the second set of nanoparticles to the total number of particles in the first set of nanoparticles on the composite material is from 1:10 to 10:1, such as from 1:5 to 5:1, such as from 2:1 to 4:1.

~~23~~17. The method according to any one of Claims ~~18~~ 13 to ~~22~~16, wherein the set of charged functional groups on the substrate layer are positively charged and the charged functional groups on the metal nanoparticle coating are negatively charged, optionally wherein:

the set of positively charged functional groups on the substrate layer are ammonium ions; and/or

the set of negatively charged functional groups on the first and second set of metal nanoparticles are carboxylate ions or alkoxide ions.

~~24. The method according to Claim 23, wherein:~~

~~the set of positively charged functional groups on the substrate layer are ammonium ions; and/or~~

~~the set of negatively charged functional groups on the first and second set of metal nanoparticles are carboxylate ions or alkoxide ions.~~

~~25~~18. The method according to any one of Claims ~~18~~ 13 to ~~22~~16, wherein the set of charged functional groups on the substrate layer are negatively charged and the charged functional groups on the metal nanoparticle coating are positively charged, optionally wherein:

the set of negatively charged functional groups on the substrate layer are carboxylate ions or alkoxide ions; and/or

the set of positively charged functional groups on the first and second set of metal nanoparticles are ammonium ions.

~~26. The method according to Claim 25, wherein:~~
~~the set of negatively charged functional groups on the substrate layer are carboxylate ions or alkoxide ions; and/or~~
~~the set of positively charged functional groups on the first and second set of metal nanoparticles are ammonium ions.~~

~~27~~19. The method according to any one of Claims ~~18~~ 13 to ~~26~~18, wherein:
the superhydrophobic material used in step (ib) of Claim 18 is a C_{10} to C_{20} thiol that is unsubstituted or substituted by one or more fluoro groups, optionally wherein the superhydrophobic material is selected from one or more of the group consisting of 1-dodecanethiol, 1-hexadecanethiol, and *1H*,*1H*,2*H*,2*H*-perfluorodecanethiol; and/or
the superhydrophobic material used to form the further superhydrophobic layer is a C_{10} to C_{20} thiol that is unsubstituted or substituted by one or more fluoro groups, optionally wherein the superhydrophobic material is selected from one or more of the group consisting of 1-dodecanethiol, 1-hexadecanethiol, and *1H*,*1H*,2*H*,2*H*-perfluorodecanethiol; and/or
the first and second set of nanoparticles are silver nanoparticles~~.~~.

~~28. The method according to any one of Claims 18 to 27, wherein the first set of metal nanoparticles are nanocubes and the second set of metal nanoparticles are nanopolyhedrons having more than six faces and are selected from one or more nanopolyhedra having from 7 to 30 faces.~~

~~29~~20. The method according to any one of Claims 13 to 19~~method according to Claim 28~~, wherein the second set of metal nanoparticles are nanooctahedra.

~~30. The method according to any one of Claims 18 to 29, wherein the first and second set of nanoparticles are silver nanoparticles.~~