provide nano-arrayed surfaces capable of physical cell destruction by mimicry of biological surfaces in an efficient and simple manner.

Nanostructures on surfaces of black silicon and TiO_2 have demonstrated microbicidal properties. However, these surface nano-patterns were generated by a top-down approach on specific materials. For example, the black silicon surface was prepared by reactive-ion beam etching on a silicon wafer. Thus, it may be appreciated that the top-down approach would become challenging when nanometer scale patterns are to be generated. In other words, top-down approaches can be time-consuming and expensive and are limited to application on surfaces of specific materials (e.g., those susceptible to etching or other forms of lithographic methods).

Hence, there is a need to provide alternative surfaces demonstrating microbicide properties that overcomes, or at least ameliorates, one or more of the disadvantages described above. There is also a need to provide simple and scalable methods to create such surfaces.

Summary

There is provided a substrate comprising a plurality of integrally formed surface features, wherein the surface features are micro-sized, nano-sized or a mixture thereof, each surface feature comprising a crystalline phase and at least one pointed terminus.

Advantageously, the surface features are integrally formed, i.e., they form a unitary body with the rest of the substrate. The formation of such surface features does not require the use of stamping techniques to transfer surface features onto the substrate surface.

Advantageously, the terminal ends of said surface features may be adapted to perturb, deform, lyse or damage cell membrane lipid layers to thereby reduce

microbe/bacteria viability or cell count. Additionally, the terminal ends may also provide a substrate surface topology that is not conducive for microbes to adhere thereon and which substantially inhibits or prevents microbial cell growth and/or reduces microbe cell count. The interaction between the microbes and surface features may be primarily or exclusively physical in nature. That is, the inhibition or killing of microbes may be achieved via non-chemical means.

There is also provided a substrate comprising a plurality of integrally formed surface features, wherein the surface features are micro-sized and/or nano-sized, each surface feature comprising a crystalline phase and at least one pointed terminus, and wherein the surface features are formed by, or obtainable from, a one-step process comprising contacting a surface of said substrate with a reagent solution comprising an alkali and an oxidizing agent to thereby integrally form the surface features on the surface of said substrate.

One aspect of the disclosure relates to a copper substrate having a plurality of ordered, integrally-formed surface structures prepared by a method comprising the steps of: contacting a surface of the copper substrate with a reagent solution, the reagent solution comprising an alkali and an oxidizing agent; wherein the oxidizing agent is selected from the group consisting of persulfates, nitrates, halogen compounds, hypohalites and permanganates; and wherein the concentration of the oxidizing agent in the reagent solution is at least about 0.3 M.

There is also provided a substrate comprising a copper surface, the copper surface comprising a plurality of surface features integrally formed thereon, said surface features being micro-sized and/or nano-sized, wherein said surface features comprises $Cu(OH)_2$, CuO or a mixture thereof, each $Cu(OH)_2$ or CuO surface feature comprising at least one pointed terminus.

There is also provided a substrate comprising a zinc surface, the zinc surface comprising a plurality of micro-sized and/or nano-

sized ZnO surface features integrally formed thereon, each ZnO surface feature comprising at least one pointed terminus.

There is also provided a method of producing a substrate possessing antibacterial properties, the method comprising: contacting a surface of the substrate with a reagent solution to produce a plurality of integrally formed, micro-sized and/or nano-sized surface features on the substrate surface, each surface feature comprising a crystalline phase and at least one pointed terminus.

There is also provided a method of producing a substrate possessing antibacterial properties, the method comprising: contacting a surface of the substrate with a reagent solution to produce a plurality of integrally formed, micro-sized or nano-sized surface features by precipitation on the substrate surface, each surface feature comprising a crystalline phase and at least one pointed terminus.

Advantageously, the disclosed methods are capable of providing the surface features having physical dimensions that are adjustable or scalable to exhibit antibacterial properties. For instance, the dimensions may be adjusted by varying the composition/concentration of the reagent solution or the contacting time. Further advantageously, the resolution of these surface features is not limited by the resolution of a mold as is the case when using conventional etching or lithography techniques to form surface features. More advantageously, the disclosed method does not require complex or multi-step nano-imprinting or screen printing methods to obtain nano-sized surface features on the surface of the substrate. Advantageously, the disclosed method can be used with “hard” metal substrates which may not be malleable to conventional surface modification techniques. The disclosed method is also capable of forming these surface features in relative short time periods compared to complex, high resolution lithography techniques (e.g., electron beam lithography). Advantageously, the disclosed method is capable of preparing metal substrates capable of bio-mimicry, e.g., replicating or simulating physical, non-chemical bacteria killing properties found in nature.

Another aspect of the disclosure relates to a method of killing or inhibiting the growth of a microorganism, said method comprising the steps of: providing a copper substrate as defined herein; and contacting said microorganism with said copper substrate.

Another aspect of the disclosure relates to a method of killing or inhibiting the growth of microorganism, said method comprising the steps of: providing a zinc substrate, said zinc substrate comprising a plurality of integrally formed needles on a surface of the zinc substrate, each needle comprising a hexagonal crystal structure and at least one pointed terminus, wherein the length of each needle is from about 500 nm to 5 μm, wherein the cross-section of the base of each needle is circular having a diameter of about 10 nm to 500 nm, wherein the distal end of the needle is a tip of circular cross-section having a diameter of about 1 nm to 100 nm, wherein the zinc surface is reactive with a nitrate to form an insoluble salt, and wherein the hexagonal crystal structure comprises the insoluble oxide salt; and contacting said microorganism with said zinc substrate.

Another aspect of the disclosure relates to a method of killing or inhibiting the growth of a microorganism, said method comprising the steps of: providing a zinc substrate having a plurality of ordered, integrally-formed surface structures prepared by: contacting a surface of the zinc substrate with a reagent solution, the reagent solution comprising an alkali and a nitrate; wherein the concentration of the alkali in the reagent solution is from about 1.0 M to about 2.5 M; contacting said microorganism with said zinc substrate.

The present disclosure further provides methods of providing antibacterial or antimicrobial properties to a surface by coupling the substrates as disclosed herein to the surface. There is further provided the non-therapeutic use of the substrates disclosed herein for inhibiting the growth of or for killing bacteria or microbes in an ex-vivo environment, e.g., for sterilizing systems, medical kits, equipment, apparel,

etc. Alternatively, the substrates as disclosed herein may also be used in therapy, e.g., wound plasters.

Another aspect of the disclosure relates to use of the copper substrate as defined herein or the zinc substrate as defined herein for providing antibacterial properties to an ex-vivo environment.

Definitions

The following words and terms used herein shall have the meaning indicated:

The term “microbe” refers to one or a plurality of microorganisms which include bacteria, fungi, algae, yeasts, molds and viruses.

The term “antimicrobial” refers to anything that kills or inhibits the growth of microbes. The term “antimicrobial” can be used to describe a thing or a characteristic of the thing and in this context, refers to the ability to kill or inhibit the growth of microbes. Accordingly, the term “antibacterial” refers to anything that kills or inhibits the growth of bacteria or, when describing a thing or a characteristic of the thing, refers to the ability to kill or inhibit the growth of bacteria. The terms “antimicrobial”, “microbicide” and “biocide” are used interchangeably.

The prefix “nano” denotes average sizes of a scale below 1 µm. Accordingly, the term “nano-sized”, as used in the context of the specification, refers to a feature having at least one dimension, e.g. length or height, with a nanoscale size. The term “nanostructure” or grammatical variants thereof is to be interpreted accordingly, to refer to a feature or pattern, e.g. blade or tube, having at least one dimension in the nanoscale.

The prefix “micro” denotes average sizes of a scale between about 1 µm to about 1000 µm. Accordingly, the term “micro-sized”, as used in the context of the specification, refers to a feature having at least one dimension, e.g. length or height, with a microscale size.

The term “crystalline” or “crystalline phase” as used herein is to be broadly interpreted to refer to a physical state having regularly repeating arrangement of

Claims

1. A copper substrate having a plurality of ordered, integrally-formed surface structures prepared by a method comprising the steps of:

contacting a surface of the copper substrate with a reagent solution, the reagent solution comprising an alkali and an oxidizing agent;

wherein the oxidizing agent is selected from the group consisting of persulfates, nitrates, halogen compounds, hypohalites and permanganates; and

wherein the concentration of the oxidizing agent in the reagent solution is at least about 0.3 M.

2. The substrate of claim 1, wherein the mole ratio of the oxidizing agent/alkali is from about 1:10 to 1:30.

3. The substrate of claim 2, wherein the mole ratio of the oxidizing agent/alkali is about 1:20.

4. The substrate of any one of claims 1-3, wherein the oxidizing agent is a persulfate.

5. The substrate of any one of claims 1-4, wherein the alkali is NaOH or KOH.

6. The substrate of any one of claims 1-5, wherein the contacting step is conducted for a duration sufficient to produce the plurality of surface features.

7. The substrate of claim 6, wherein the contacting step is conducted for a duration of from about 25 minutes to about 35 minutes.

8. The substrate of any one of claims 1-7, wherein contacting step comprises dipping said substrate into said reagent solution.

9. The substrate of any one of claims 1-8, wherein the surface structure comprises a length of from about 200 nm to 5 μm, breadth of from about 100 nm to 500 nm, said surface structure having a thickness which tapers from the surface of the substrate towards the distal end of the blade, wherein the thickness of the blade at the distal end is about 10 nm to 30 nm.

10. The substrate of any one of claims 1-9, wherein the surface of the copper substrate is reactive with the oxidizing agent to form an insoluble salt.

11. The substrate of any one of claims 1-10, wherein the surface structure comprises a monoclinic crystal structure.

12. The substrate of claim 11, wherein said monoclinic crystal structure comprises an oxide salt.

13. A method of killing or inhibiting the growth of a microorganism, said method comprising the steps of:

providing a copper substrate of any one of claims 1-12; and

contacting said microorganism with said copper substrate.

14. A method of killing or inhibiting the growth of microorganism, said method comprising the steps of:

providing a zinc substrate,

said zinc substrate comprising a plurality of integrally formed needles on a surface of the zinc substrate, each needle comprising a hexagonal crystal structure and at least one pointed terminus, wherein the length of each needle is from about 500 nm to 5 μm, wherein the cross-section of the base of each needle is circular having a diameter of about 10 nm to 500 nm,

wherein the distal end of the needle is a tip of circular cross-section having a diameter of about 1 nm to 100 nm,

wherein the zinc surface is reactive with a nitrate to form an insoluble salt, and

wherein the hexagonal crystal structure comprises the insoluble oxide salt; and

contacting said microorganism with said zinc substrate.

15. A method of killing or inhibiting the growth of a microorganism, said method comprising the steps of:

providing a zinc substrate having a plurality of ordered, integrally-formed surface structures prepared by:

contacting a surface of the zinc substrate with a reagent solution, the reagent solution comprising an alkali and a nitrate;

wherein the concentration of the alkali in the reagent solution is from about 1.0 M to about 2.5 M;

contacting said microorganism with said zinc substrate.

16. The method of any one of claims 13-15, wherein the microorganism is gram-negative or gram-positive bacteria.

17. The method of claim 16, wherein the gram-negative bacteria is selected from the group consisting of Escherichia, Shigella, and Salmonella.

18. The method of claim 16, wherein the gram-positive bacteria is selected from the group consisting of Staphylococcus, Enterococcus and Streptococcus.

19. Use of the copper substrate of any one of claims 1-12 or the zinc substrate as defined in claim 14 or 15 for providing antibacterial properties to an ex-vivo environment.

20. The use of claim 19 for providing bacteriostatic or bactericidal purposes to said ex-vivo environment.

21. The use of claims 19 or 20, being a non-therapeutic use.

22. The use of any one of claims 19-21, wherein the antibacterial substrate is capable of killing or inhibiting the growth of gram-negative and gram-positive bacteria.

23. The use of claim 22, wherein the gram-negative bacteria is selected from the group consisting of Escherichia, Shigella, and Salmonella.

24. The use of claim 22, wherein the gram-positive bacteria is selected from the group consisting of Staphylococcus, Enterococcus and Streptococcus.