

Description

Title of Invention: Substrate Coated With Layered Double Hydroxides

Technical Field

- 5 The present invention generally relates to a substrate coated with a layered double hydroxide having antimicrobial properties. The present invention also relates to a method of preparing the substrate, and the use of calcined or uncalcined layered double hydroxide as an antimicrobial.

Background Art

- 10 Infectious diseases caused by pathogens are a major threat to human health. Pathogens have the ability to adhere to and multiply on surfaces over long periods of time, thus, diseases may be spread indirectly when contacting shared surfaces, particularly high touch surfaces or in areas of high human traffic. In a healthcare setting, infections could also arise from contaminated implants.

- 15 To combat the spread of pathogens through surfaces, coatings which inhibit the growth of and/or kill microbes have been developed. One class of coatings incorporate antimicrobial chemicals such as antibiotics in the material and act by releasing the chemical over time, thereby killing or preventing the continued growth of microbes. However, the widespread use of such antimicrobial chemicals could lead to the generation of superbugs or chemically
20 resistant bacteria, which is a significant problem.

- Recently, there has been great interest in nanostructures capable of inducing mechanical or physical damage to microbes upon contact, which leads to cell rupture and death. Owing to the membrane-lytic nature of the mechanism, the likelihood of microbes developing drug resistance from such nanostructure related antimicrobial coatings is low. Examples such as
25 artificial nanopillar arrays of black silicon, ZnO, ZIF-L and other similar structures have been reported, which display antibacterial activity. However, there is a lack of general methods to make nanostructure materials from inexpensive building blocks which are also biocompatible.

- 30 Layered double hydroxides (LDH or anionic clays) are an important class of materials. They consist of positively charged metal hydroxide sheets with intercalated anions and water molecules. LDH is made from common elements and may be tuned for different purposes by changing their component metal ions and anions. An important feature of LDH is its excellent biocompatibility, thus there have been many reported biomedical applications of LDH such as in drug delivery or bioimaging.

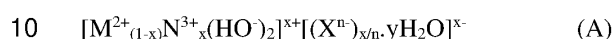
- 35 LDH exhibiting antimicrobial activity have been reported, but only when used in combination with antimicrobial chemicals such as Ag nanoparticles or antibiotics, whereby the LDH effectively merely acts as a carrier of the antimicrobial chemicals. In such instances,

the mechanism of action is through chemical interaction of the antimicrobials with the microbes, which may lead to drug resistant microbes.

- There is therefore a need to provide a substrate that overcomes, or at least ameliorates, one or more of the disadvantages described above. Specifically, there is a need to provide a material that is biocompatible, inexpensive and does not give rise to drug resistance in microbes.

Summary

According to a first aspect, there is provided a substrate coated with a layered double hydroxide having the following structure (A):



wherein

M^{2+} is a divalent cation;

N^{3+} is a trivalent cation;

x is in the range of 1/6 to 1/2;

- 15 y is in the range of 0.5 to 4;

X^{n-} is an anion; and

n is an integer selected from 1, 2 or 3;

- wherein the layered double hydroxide is surface modified with a group 10 element on the Periodic Table of Elements or a compound selected from the group consisting of: a fatty acid, a polymer, and an amino acid.

- Advantageously, the substrate coated with the layered double hydroxide (LDH) as disclosed herein may act as an antimicrobial even in the absence of antimicrobial chemicals. More advantageously, the layered double hydroxide (LDH) may be surface modified with a non-antimicrobial group 10 element on the Periodic Table of Elements or a non-antimicrobial compound selected from the group consisting of: a fatty acid, a polymer, and an amino acid to confer antimicrobial properties to a surface.

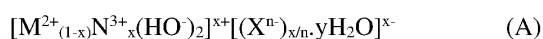
- Advantageously, the surface-modification of the LDH with a non-antimicrobial compound may alter the hydrophilicity of the LDH, which may in turn confer the antimicrobial properties to the LDH. Advantageously, this may overcome the limitations of conventional antimicrobial chemicals of developing drug resistance.

Further advantageously, non-surface-modified vertically aligned LDH (V-LDH) array may have very weak antimicrobial property. However, the surface modified V-LDH array may

advantageously exhibit excellent antimicrobial properties. Further advantageously, LDH may have excellent biocompatibility.

In another aspect, there is provided a method of preparing a substrate coated with a surface-modified layered double hydroxide, the method comprising the steps of:

- 5 providing a substrate coated with a layered double hydroxide having the following structure (A):



wherein

M^{2+} is a divalent cation;

N^{3+} is a trivalent cation;

- 10 x is in the range of 1/6 to 1/2;

y is in the range of 0.5 to 4;

X^{n-} is an anion; and

n is an integer selected from 1, 2 or 3; and

- 15 contacting the substrate coated with a layered double hydroxide with a group 10 element on the Periodic Table of Elements or a compound selected from the group consisting of: a fatty acid, a polymer, and an amino acid.

In an example, the substrate coated with the layered double hydroxide may be prepared by contacting a substrate with a compound having a formula $M^+(X^*)_2\cdot zH_2O$ and a compound having a formula $N^+(X^*)_3\cdot zH_2O$ in the presence of a base,

- 20 wherein M^+ is a divalent cation;

N^+ is a trivalent cation;

z is independently in the range of 1 to 12;

X^* is independently an anion; and

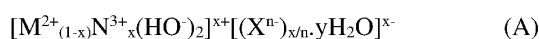
heating the substrate.

- 25 Advantageously, the surface modified layered double hydroxide may be prepared by simply combining two inexpensive metal salts with a base such as urea and the chemicals used for surface modification may be inexpensive and readily available. Furthermore, the method is scalable.

More advantageously, the double layered hydroxide may be prepared in water, without the need for organic solvents, which is environmentally friendly.

Further advantageously, the modified layered double hydroxide may be coated on a variety of substrates such as glass, plastics, metals, ceramics, silicone and any mixture thereof. This makes the process versatile, with the ability to be adapted to a variety of applications. More advantageously, the coating may adhere strongly to the substrate.

- 5 In another aspect, there is provided the use of a layered double hydroxide as an antimicrobial, the layered double hydroxide having the following structure (A):



wherein

M^{2+} is a divalent cation;

- 10 N^{3+} is a trivalent cation;

x is in the range of 1/6 to 1/2;

y is in the range of 0.5 to 4;

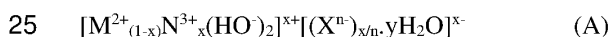
X^{n-} is an anion; and

n is an integer selected from 1, 2 or 3;

- 15 wherein the layered double hydroxide is surface modified with a group 10 element on the Periodic Table of Elements or a compound selected from the group consisting of: a fatty acid, a polymer, and an amino acid.

- Advantageously, surface modification may alter the hydrophilicity of the layered double hydroxide, thereby resulting in a reduction in microbial count on the surface. Advantageously, 20 the surface modified layered double hydroxide may achieve up to 7 log reduction of bacteria. Further advantageously, surface modified layered double hydroxide may have good blood clearance and may be well absorbed by the human body, making them extremely biocompatible.

In another aspect, there is provided the use of a calcined layered double hydroxide as an antimicrobial, the layered double hydroxide having the following structure (A):



wherein

M^{2+} is a divalent cation;

N^{3+} is a trivalent cation;

x is in the range of 1/6 to 1/2;

- 30 y is in the range of 0.5 to 4;

X^{n-} is an anion; and

n is an integer selected from 1, 2 or 3.

Advantageously, the calcined layered double hydroxide may have antimicrobial properties even without any surface modification.

5 Definitions

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

10 The term ‘vertically aligned’, for the purposes of this disclosure, is to be interpreted broadly to refer to the orientation of an individual layered double hydroxide piece with respect to the surface it is on. An LDH is said to be vertically aligned if its longitudinal axis is approximately perpendicular to the surface. Approximately perpendicular means to have an angle, relative to the surface it is on, in the range of about 85° to about 95°, more preferably in the range of about 88° to 92°. A group of LDH is said to be vertically aligned if at least 70% of the LDH in the group are vertically aligned.

15 The word “substantially” does not exclude “completely” e.g. a composition which is “substantially free” from Y may be completely free from Y. Where necessary, the word “substantially” may be omitted from the definition of the invention.

Unless specified otherwise, the terms "comprising" and "comprise", and grammatical variants thereof, are intended to represent "open" or "inclusive" language such that they include recited elements but also permit inclusion of additional, unrecited elements.

20 As used herein, the term "about", in the context of concentrations of components of the formulations, typically means +/- 5% of the stated value, more typically +/- 4% of the stated value, more typically +/- 3% of the stated value, more typically, +/- 2% of the stated value, even more typically +/- 1% of the stated value, and even more typically +/- 0.5% of the stated value.

25 Throughout this disclosure, certain embodiments may be disclosed in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the disclosed ranges. Accordingly, the description of a range should be considered to have specifically disclosed all the possible sub-ranges as well as individual numerical values within that range.

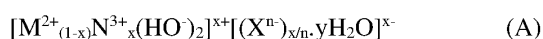
30 For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed sub-ranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 3, 4, 5, and 6. This applies regardless of the breadth of the range.

35 Certain embodiments may also be described broadly and generically herein. Each of the narrower species and subgeneric groupings falling within the generic disclosure also form part of the disclosure. This includes the generic description of the embodiments with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein.

Detailed Disclosure of Embodiments

Exemplary, non-limiting embodiments of surface modified layered double hydroxide coatings, will now be disclosed.

- 5 There is provided a substrate coated with a layered double hydroxide having the following structure (A):



wherein

M^{2+} is a divalent cation;

N^{3+} is a trivalent cation;

- 10 x is in the range of 1/6 to 1/2;

y is in the range of 0.5 to 4;

X^{n-} is an anion; and

n is an integer of 1 to 3;

- 15 wherein the layered double hydroxide is surface modified with a group 10 element on the Periodic Table of Elements or a compound selected from the group consisting of: a fatty acid, a polymer, and an amino acid.

Layered double hydroxides consist of positively charged metal hydroxide sheets with intercalated anions and water molecules. They have a general formula represented by formula (A) above, and is highly flexible on its composition being tuned.

- 20 The layered double hydroxide may be calcined.

- 25 M^{2+} may be a divalent cation. M^{2+} may be selected from a group 2, group 7, group 8, group 9, group 10, group 11 or group 12 element on the Periodic Table of Elements. M^{2+} may be Mg^{2+} , Ca^{2+} , Sr^{2+} , Ba^{2+} , Mn^{2+} , Tc^{2+} , Re^{2+} , Fe^{2+} , Ru^{2+} , Co^{2+} , Rh^{2+} , Ir^{2+} , Ni^{2+} , Pd^{2+} , Pt^{2+} , Cu^{2+} , Au^{2+} , Ag^{2+} , Zn^{2+} , Cd^{2+} or Hg^{2+} . M^{2+} may be selected from the group consisting of Mg^{2+} , Zn^{2+} , Cu^{2+} , Ni^{2+} and Co^{2+} .

N^{3+} may be a trivalent cation. N^{3+} may be selected from a group 6, group 8, or group 13 element on the Periodic Table of Elements. N^{3+} may be Cr^{3+} , Mo^{3+} , W^{3+} , Fe^{3+} , Ru^{3+} , Os^{3+} , Al^{3+} , Ga^{3+} , or In^{3+} . N^{3+} may be selected from the group consisting of Al^{3+} , Fe^{3+} and Cr^{3+} .

- 30 The stoichiometric $M^{2+}:N^{3+}$ ratio may be in the range of 1:1 to 5:1. The stoichiometric $M^{2+}:N^{3+}$ ratio may be in the range of 2:1 to 5:1, 3:1 to 5:1, 4:1 to 5:1, 1:1 to 4:1, 2:1 to 4:1, 3:1 to 4:1, 1:1 to 3:1, 2:1 to 3:1, or 1:1 to 2:1.

x may be in the range of 1/6 to 1/2. x may be in the range of 1/5 to 1/2, 1/4 to 1/2, 1/3 to 1/2, 1/6 to 1/3, 1/5 to 1/3, 1/4 to 1/3, 1/6 to 1/4, 1/5 to 1/4, or 1/6 to 1/5.

y may be in the range of 0.5 to 4. y may be in the range of 1 to 4, 1.5 to 4, 2 to 4, 2.5 to 4, 3 to 4, 0.5 to 3, 1 to 3, 1.5 to 3, 2 to 3, 2.5 to 3, 0.5 to 2, 1 to 2, 1.5 to 2, or 0.5 to 1.

The layered double hydroxide may have different H₂O content from sample to sample.

- 5 X^{n-} is the intercalating anion (or anions) that are intercalated with the positively charged metal hydroxide sheets in the layered double hydroxide. They are weakly bound to the metal hydroxide sheets, and may be replaced with other anions easily.

X^{n-} may be NO_3^- , Cl^- , Br^- , OH^- , CO_3^{2-} , SO_4^{2-} , ClO_4^- , benzoate or terephthalate. X^{n-} may be NO_3^- , Cl^- , CO_3^{2-} or SO_4^{2-} .

n may be an integer selected from 1, 2 or 3.

- 10 X^{n-} may be NO_3^- or Cl^- and n may be 1, or X^{n-} may be SO_4^{2-} and n may be 2.

The layered double hydroxide may be vertically aligned, with respect to the substrate where it is coated on.

Each piece of the layered double hydroxide may have a hexagonal shape.

- 15 The layered double hydroxide may have an average thickness in the range of about 50 nm to about 175 nm. The layered double hydroxide may have an average thickness in the range of about 100 nm to about 175 nm, about 150 nm to about 175 nm, about 50 nm to about 150 nm, about 100 nm to about 150 nm or about 50 nm to about 100 nm.

- 20 The layered double hydroxide may have an average spacing between each piece of adjacent LDH in the range of about 300 nm to about 550 nm. Each piece of the LDH may have an edge, whereby the edge may refer to the farthest perpendicular point of the LDH with respect to the surface of the substrate it is growing on (as indicated by (102) in Fig. 1), and the average spacing may refer to the spacing between the edge of each piece of adjacent LDH. The layered double hydroxide may have an average spacing between each piece of adjacent LDH in the range of about 350 nm to about 550 nm, about 400 nm to about 550 nm, about 25 450 nm to about 550 nm, about 500 nm to about 550 nm, about 300 nm to about 450 nm, about 350 nm to about 450 nm, about 400 nm to about 450 nm or about 300 nm to about 350 nm.

- 30 When the layered double hydroxide is calcined, the calcined layered double hydroxide may have an average thickness in the range of about 50 nm to about 100 nm. The calcined layered double hydroxide may have an average thickness in the range of about 60 nm to about 100 nm, about 80 nm to about 100 nm, about 50 nm to about 90 nm, about 60 nm to about 90 nm, about 80 nm to about 90 nm, about 50 nm to about 80 nm, about 60 nm to about 80 nm, about 50 nm to about 70 nm, about 60 nm to about 70 nm or about 50 nm to about 60 nm.

- 35 The fatty acid may be a C_{15} to C_{22} fatty acid. The fatty acid may be a C_{16} to C_{22} fatty acid, C_{18} to C_{22} fatty acid, C_{20} to C_{22} fatty acid, C_{15} to C_{20} fatty acid, C_{16} to C_{20} fatty acid, C_{18} to C_{20} fatty acid, C_{15} to C_{18} fatty acid or C_{17} to C_{18} fatty acid. The fatty acid may be oleic acid, linoleic acid, erucic acid, or elaidic acid. The fatty acid may be oleic acid.

The polymer may be a polymer comprising an amine group. The polymer may be polyethyleneimine, polypropyleneimine, polybutyleneimine, poly(*N*-methylvinylamine), poly(4-aminostyrene), or polyallylamine. The polymer may be polyethyleneimine. The polymer may be branched or linear.

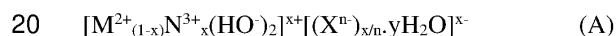
- 5 The amino acid may be an amino acid having a charged side chain. The amino acid may be L-glutamic acid, L-arginine, L-aspartic acid, L-histidine or L-lysine. The amino acid may be L-glutamic acid or L-arginine.

- 10 The group 10 element on the Periodic Table of Elements may be selected from the group consisting of nickel, palladium and platinum. The group 10 element on the Periodic Table of Elements may be platinum.

- 15 The substrate may be selected from the group consisting of glass, plastic, metal or alloy, ceramic, silicone and any mixture thereof. The plastic may be poly(methyl methacrylate) (PMMA), polytetrafluoroethylene (PTFE), polycarbonate or polyethylene. The metal may be titanium, aluminium, gold, silver or iron. The metal alloy may be stainless steel, steel, white gold, titanium alloy or cast iron. The ceramic may be porcelain or brick.

There is also provided a method of preparing a substrate, the method comprising the steps of:

providing a substrate coated with a layered double hydroxide having the following structure (A):



wherein

M^{2+} is a divalent cation;

N^{3+} is a trivalent cation;

x is in the range of 1/6 to 1/2;

- 25 y is in the range of 0.5 to 4;

X^{n-} is an anion; and

n is an integer selected from 1, 2 or 3; and

- 30 contacting the substrate coated with a layered double hydroxide with a group 10 element on the Periodic Table of Elements or a compound selected from the group consisting of: a fatty acid, a polymer, and an amino acid.

- 35 The contacting of the substrate coated with a layered double hydroxide with a group 10 element on the Periodic Table of Elements or a compound selected from the group consisting of: a fatty acid, a polymer, and an amino acid, to surface modify the layered double hydroxide, may change the hydrophilicity of the layered double hydroxide such that its contact angle with water may be about 0 °C.

The layered double hydroxide may be calcined before contacting the substrate with the group 10 element on the Periodic Table of Elements or the compound selected from the group consisting of: a fatty acid, a polymer, and an amino acid.

- 5 The calcining step may be performed in air at a temperature in the range of about 400 °C to about 500 °C. The calcining step may be performed in air at a temperature in the range of about 425 °C to about 500 °C, about 450 °C to about 500 °C, about 475 °C to about 500 °C, about 400 °C to about 475 °C, about 425 °C to about 475 °C, about 450 °C to about 475 °C, about 400 °C to about 450 °C, about 425 °C to about 450 °C or about 400 °C to about 425 °C.

- 10 The calcining step may be performed for a duration in the range of about 40 hours to about 60 hours. The calcining step may be performed for a duration in the range of about 45 hours to about 60 hours, about 50 hours to about 60 hours, about 55 hours to about 60 hours, about 40 hours to about 55 hours, about 45 hours to about 55 hours, about 50 hours to about 55 hours, about 40 hours to about 50 hours, about 45 hours to about 50 hours or about 40 hours to about 45 hours.

- 15 The substrate coated with the layered double hydroxide may be prepared by contacting a substrate with a compound having a formula $M'(X')_2 \cdot zH_2O$ and a compound having a formula $N'(X')_3 \cdot zH_2O$ in the presence of a base,

wherein M' is a divalent cation;

N' is a trivalent cation;

- 20 z is independently in the range of 1 to 12;

X' is independently an anion; and

heating the substrate.

- 25 z may be in the range of 1 to 12. z may be in the range of 1 to 10, 1 to 8, 1 to 6, 1 to 4, 1 to 2, 3 to 12, 3 to 10, 3 to 8, 3 to 6, 3 to 4, 5 to 12, 5 to 10, 5 to 8, 5 to 6, 7 to 12, 7 to 10, 7 to 8, 9 to 12, 9 to 10 or 11 to 12.

- 30 M' may be a divalent cation. M' may be selected from a group 2, group 7, group 8, group 9, group 10, group 11 or group 12 element on the Periodic Table of Elements. M' may be Mg^{2+} , Ca^{2+} , Sr^{2+} , Ba^{2+} , Mn^{2+} , Tc^{2+} , Re^{2+} , Fe^{2+} , Ru^{2+} , Co^{2+} , Rh^{2+} , Ir^{2+} , Ni^{2+} , Pd^{2+} , Pt^{2+} , Cu^{2+} , Au^{2+} , Ag^{2+} , Zn^{2+} , Cd^{2+} or Hg^{2+} . M' may be selected from the group consisting of Mg^{2+} , Zn^{2+} , Cu^{2+} , Ni^{2+} and Co^{2+} .

N' may be a trivalent cation. N' may be selected from a group 6, group 8, or group 13 element on the Periodic Table of Elements. N' may be Cr^{3+} , Mo^{3+} , W^{3+} , Fe^{3+} , Ru^{3+} , Os^{3+} , Al^{3+} , Ga^{3+} , or In^{3+} . N' may be selected from the group consisting of Al^{3+} , Fe^{3+} and Cr^{3+} .

- 35 The stoichiometric $M':N'$ ratio may be in the range of 1:1 to 5:1. The stoichiometric $M':N'$ ratio may be in the range of 2:1 to 5:1, 3:1 to 5:1, 4:1 to 5:1, 1:1 to 4:1, 2:1 to 4:1, 3:1 to 4:1, 1:1 to 3:1, 2:1 to 3:1, or 1:1 to 2:1.

The $M':N'$ ratio may be 2:1.

X' may be NO_3^- , Cl^- , Br^- , OH^- , CO_3^{2-} , SO_4^{2-} , ClO_4^- , benzoate or terephthalate. X' may be NO_3^- , Cl^- , CO_3^{2-} or SO_4^{2-} .

$M'(X')_2 \cdot y\text{H}_2\text{O}$ may be $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ or $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ and $N'(X')_3 \cdot y\text{H}_2\text{O}$ may be $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$, $\text{Cr}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$, $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ or $\text{InCl}_3 \cdot 4\text{H}_2\text{O}$.

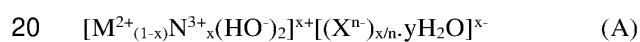
5 $M'(X')_2 \cdot y\text{H}_2\text{O}$ may be $\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ and $N'(X')_3 \cdot y\text{H}_2\text{O}$ may be $\text{Al}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$.

The preparation may be performed in the presence of a base. The base may be selected from hexamethylenetetramine, potassium hydroxide, sodium hydroxide or ammonium hydroxide. The preparation may be performed in the presence of urea.

10 The heating step may be performed at a temperature in the range of about 80 °C to about 110 °C. The heating step may be performed at a temperature in the range of about 80 °C to about 100 °C, about 80 °C to about 90 °C, about 90 °C to about 110 °C, about 90 °C to about 100 °C or about 100 °C to about 110 °C.

15 The heating step may be performed for a duration in the range of about 40 hours to about 60 hours. The heating step may be performed for a duration in the range of about 45 hours to about 60 hours, 50 hours to about 60 hours, 55 hours to about 60 hours, 40 hours to about 55 hours, 45 hours to about 55 hours, 50 hours to about 55 hours, 40 hours to about 50 hours, 45 hours to about 50 hours or 40 hours to about 45 hours.

There is also provided use of a layered double hydroxide as an antimicrobial, the layered double hydroxide having the following structure (A):



wherein

M^{2+} is a divalent cation;

N^{3+} is a trivalent cation;

x is in the range of 1/6 to 1/2;

25 y is in the range of 0.5 and 4;

X^{n-} is an anion; and

n is an integer of 1 to 3;

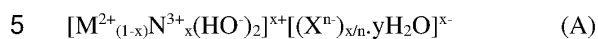
30 wherein the layered double hydroxide is surface modified with a group 10 element on the Periodic Table of Elements or a compound selected from the group consisting of: a fatty acid, a polymer, and an amino acid.

The layered double hydroxide may be calcined.

35 The antimicrobial may inhibit the growth of a microorganism, or may prevent an infection caused by a microorganism. The microorganism may be a bacteria or fungi. The microorganism may be *E. coli*, *S. aureus*, methicillin-resistant *S. aureus*, *C. albicans*, *C. difficile*, *K. pneumoniae*, *B. cereus*, *S. enteritidis* or *P. aeruginosa*. The microorganism may

be selected from the group consisting of *E. coli*, *S. aureus*, Methicillin-resistant *S. aureus* and *C. albicans*.

There is also provided the use of a calcined layered double hydroxide as an antimicrobial, the layered double hydroxide having the following structure (A):



wherein

M^{2+} is a divalent cation;

N^{3+} is a trivalent cation;

x is in the range of 1/6 to 1/2;

10 y is in the range of 0.5 to 4;

X^{n-} is an anion; and

n is an integer of 1 to 3

Brief Description of Drawings

15 The accompanying drawings illustrate a disclosed embodiment and serves to explain the principles of the disclosed embodiment. It is to be understood, however, that the drawings are designed for purposes of illustration only, and not as a definition of the limits of the invention.

Fig.1

20 [Fig. 1] refers to Scanning Electron Microscope (SEM) images of (a) as prepared V-LDH on a glass substrate and (b) V-LDH on a glass substrate after calcination at 450 °C for 18 hours. Bar = 1µm

Fig.2

[Fig. 2] refers to X-ray diffraction (XRD) patterns of samples of V-LDH on a glass substrate before (202) and after (204) calcination.

25 Fig.3

[Fig. 3] refers to SEM images of V-LDH on (a) polycarbonate, (b) stainless steel and (c) ceramic. Bar = 1µm

Fig.4

[Fig. 4] refers to an XRD pattern of V-LDH on a polycarbonate surface.

Fig.5

[Fig. 5] refers to SEM images showing (a) *E. coli*, (b) *S. aureus* and (c) *C. albicans* cells on samples of calcined V-LDH glass surface, which were ruptured and killed after incubation for 24 hours. Bar = 1 μ m

5 Fig.6

[Fig. 6] refers to graphs showing Japan Industrial Standard (JIS) testing results of as prepared and calcined V-LDH on glass with (a) *E. coli* and (b) *S. aureus*.

Fig.7

[Fig. 7] refers to an SEM image of calcined V-LDH on titanium. Bar = 1 μ m

10 Fig.8

[Fig. 8] refers to a graph showing JIS testing results of V-LDH on titanium with MRSA.

Examples

Non-limiting examples of the invention and a comparative example will be further described in greater detail by reference to specific Examples, which should not be construed as in any way limiting the scope of the invention.

Example 1: Materials and Methods**Materials**

Mg(NO₃)₂·6H₂O and Al(NO₃)₃·6H₂O were purchased from Merck, Singapore, and urea was purchased from Bio-RAD, California, United States of America (USA). Glass slides were purchased from Marienfeld, Germany. Oleic acid, polyethylenimine (PEI, branched, average Mw ~25,000), L-arginine and L-glutamic acid were purchased from Sigma-Aldrich, Singapore. Tryptic soy broth (TSB) and yeast mould broth (YMB) powder were purchased from BD Diagnostics, Singapore and used to prepare the broths according to the manufacturer's instructions. Gram-negative bacteria *E. coli* (ATCC No. 8739), gram-positive bacteria *S. aureus* (ATCC No. 6538P), and fungi *C. albicans* (ATCC No. 10231) were purchased from American Type Culture Collection (ATCC), Virginia, USA and re-cultured according to the protocols described in the sub-section "*Bacterial culture*" below. MRSA (*S. aureus* MRSA-R10309N) was acquired from Tan Tock Seng Hospital (TTSH), Singapore. Implant foil Ti90/Al6/V4 and Titanium (>99.6%) were purchased from Goodfellow, Huntingdon, United Kingdom.

Characterization of surface

The surfaces of the samples were characterized by Scanning Electron Microscopy (SEM) (JEOL JSM-7400E) and X-Ray Diffraction (XRD) (PANalytical X-ray diffractometer, X'pert PRO, with Cu K α radiation at 1.5406 Å). Prior to SEM, the samples were coated with thin Pt film using high resolution sputter coater (JEOL, JFC-1600 Auto Fine Coater). Coating conditions: (20 mA, 30 s). The samples were examined by X-ray diffraction (XRD). Contact angle measurement was from OCA20 contact angle measuring device, Dataphysics

Instruments, Germany. Zeta potential measurement was collected from Zetasizer Nano ZS, Malvern Instruments, UK. Ultrasonic (VMR Model 50 HT, 50/60 HZ).

Bacterial culture

5 *E. coli*, *S. aureus* and Methicillin-resistant *S. aureus* (MRSA) were cultured in TSB overnight in an incubator at 37 °C under constant shaking (300 rpm). *C. albicans* were grown in YMB at room temperature under constant shaking (300 rpm). Prior to each bacterial experiment, bacteria/fungus was refreshed from stock to 5 ml of respective nutrient broth. Cells were collected at the logarithmic stage of growth and the suspensions were adjusted to OD₆₀₀ = 0.07 using TSB or YMB. This yielded a microbial stock solution with ~3 × 10⁸ colony forming units (CFU) per mL.

JIS Z 2801 method for killing efficacy testing

15 *E. coli* were suspended in 5 mL of 1/500 TSB nutrient broth and adjusted to OD₆₀₀ = 0.07, corresponding to 3 × 10⁸ CFU/ml. The solution was further diluted 100 times with 1/500 TSB to obtain a bacterial count of 1~4 × 10⁵ cells/ 0.1 ml. 100 µL of cell suspensions was placed on the surfaces. Experiments were carried out in triplicate at 37 °C. After incubation with the surfaces, the respective cell suspensions were washed with 9.9 ml of TSB or YMB and diluted, and then plated on 1.5% LB agar plates. The plates were incubated for 24 hours at 37 °C. Microbial colonies were formed and counted. The resulting colonies were then counted using standard plate counts techniques, and the number of colony forming units per mL was calculated.

25 *C. albicans* were suspended in 5 mL of 1/50 YMB nutrient broth and adjusted to OD₆₀₀ = 0.07, corresponding to 3 × 10⁸ CFU/ml. The solution was further diluted 100 times with 1/50 YMB to obtain a bacterial count of 1~4 × 10⁵ cells/ 0.1 ml. 100 µL of cell suspensions was placed on the surfaces. Experiments were carried out in triplicate at 37 °C with LDH on glass. After incubation with the surfaces for 24 hours at room temperature, the respective cell suspensions were washed with 9.9 ml of YMB and diluted, and then plated on 1.5% LB agar plates. The plates were incubated for 24 hours at room temperature (25°C). Microbial colonies were formed and counted. The resulting colonies were then counted using standard plate counts techniques, and the number of colony forming units per mL was calculated.

30 MRSA were suspended in 5 mL of 1/500 TSB nutrient broth and adjusted to OD₆₀₀ = 0.07, corresponding to 3 × 10⁸ CFU/ml. The solution was further diluted 100 times with 1/100 TSB to obtain a bacterial count of 1~4 × 10⁵ cells/ 0.1 ml. 32 µL of cell suspensions was placed on the surfaces. Experiments were carried out in triplicate at 37 °C with titanium as control and LDH on titanium for testing. After incubation with the surfaces for 18-24 hours at 37 °C, the respective cell suspensions were washed with 3.2 ml of TSB and diluted, and then plated on 1.5% LB agar plates. The plates were incubated for 24 hours at 37 °C. Microbial colonies were formed and counted. The resulting colonies were then counted using standard plate counts techniques, and the number of colony forming units per mL was calculated.

SEM imaging

40 *E. coli*, *S. aureus*, and *C. albicans* were selected for bacteria adhesion study. The bacteria concentration in 1/500 TSB or 1/50 YMB (for *C. albicans*) was adjusted to OD₆₀₀=0.07 on a microplate reader (TECAN, Switzerland), which corresponded to ~10⁸ CFU/mL. After 10²

times dilution, 100 μ L of bacteria suspension was added to the LDH surface (2.5 cm $\times$ 2.5 cm), and incubated at 37 $^{\circ}$ C (room temperature for *C. albicans*) for 24 hours.

5 The surfaces were fixed in 2.5% glutaldehyde phosphate buffered saline (PBS) solution for 2 hours, then each sample was soaked in 30%, 50%, 70%, 85%, 90% ethanol, and 100% ethanol twice for 20 minutes per concentration level. The treated surface was placed in a fume hood and left for 24-48 hours, after which it was further coated with platinum before SEM imaging. The morphologies of the bacteria before and after treatment were observed using a field emission SEM (JEOL JSM-7400F) operated at an accelerating voltage of 10.0 kV and working distance of 8.0 mm.

10 Example 2: Surface Coating

In an example, vertically aligned LDH (V-LDH) was prepared on a glass surface. An aqueous solution of $\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ (1.71 g), $\text{Al}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ (1.25 g) ($\text{Mg} : \text{Al} = 2 : 1$), and urea (5.6 g) in 100 ml water was placed in a 100 ml KIMAX glass bottle. The substrate surface (2.5 cm $\times$ 2.5 cm glass) was washed with a solution of ethanol/deionized water, before being submerged into the bottle and placed horizontally flat at the bottom with the growing surface facing upwards. The bottle was heated in an oven at 90 $^{\circ}$ C for about 48 hours. The surface was then washed with deionized water and ethanol, and dried in ambient air. SEM imaging showed that the whole surface was densely coated with closely packed vertically aligned LDH (Fig. 1a). Each piece of LDH was a hexagonal plate with an average thickness of 157 nm, and an average spacing between each piece of adjacent LDH of 450 nm. Each piece of the LDH may have an edge (102), whereby the edge is the farthest perpendicular point of the LDH with respect to the surface of the substrate it is growing on, as indicated in Fig. 1. The average spacing refers to the spacing between the edge of each piece of adjacent LDH. It was also observed from SEM imaging of the surface that the vertically aligned LDH was growing on another flat coating of LDH, rather than on the bare glass surface. It appears that the LDH nanocrystals first attach to the substrate randomly to form the “foundation” and subsequently the V-LDH film grows on top of it. The average loading mass of LDH on one side of the 2.5 cm $\times$ 2.5 cm glass substrate was 0.24 mg.

Comparative Example 1: Non-Aligned LDH

30 In a comparative example, non-aligned LDH was prepared on a glass surface. Approximately 0.00025g of LDH Powder and 100 μ L of ethanol were added into a 1.5 ml centrifuge tube. The mixture was subjected to an ultrasonic treatment to achieve a homogenous suspension. The suspension was spread onto 2.5 cm $\times$ 2.5 cm clean glass slides. After the ethanol evaporated, free LDH coated glass slides were obtained. The surfaces were further dried in a 35 60 $^{\circ}$ C oven overnight before JIS testing.

Example 3: Calcination

The V-LDH grown on glass was calcined in air at 450 $^{\circ}$ C for 18 hours. Fig. 1b shows the SEM image of the resulting calcined V-LDH glass surface. The thickness of the LDH sheet shrank from 157 nm before calcination to 70 nm after calcination. Fig. 2 shows the XRD patterns of samples before (202) and after (204) calcination. Before calcination, the surface charge was +5.9 mV, and the contact angle was 59 $^{\circ}$. However, after calcination, the surface

charge changed to -19.5 mV, and the surface became highly hydrophilic with a contact angle of $\sim 0^\circ$.

Example 4: Surface Modification

5 The as prepared V-LDH and calcined V-LDH on glass were modified by coating with different reagents in order to study the effects of these modifications on the hydrophilicity, surface charge, and antimicrobial properties.

Modification with oleic acid or polyethylenimine (PEI)

10 1 g oleic acid or PEI (branched, average Mw $\sim 25,000$) was dissolved in 50 ml ethanol. Samples of V-LDH on glass (as prepared or calcined) were placed in the solution, with the LDH surface facing up. After 2 hours, the samples were removed from the solution, washed 3 times with ethanol, and dried at 60 °C in an oven.

Modification with Pt

15 Samples of V-LDH on glass (as prepared or calcined) were coated with a thin Pt film using high resolution sputter coater (JEOL, JFC-1600 Auto Fine Coater). Coating conditions: (20 mA, 30 s).

Modification with amino acid

20 1 g amino acid (L-arginine or L-glutamic acid) were dissolved in 50 ml water. Samples of V-LDH on glass (calcined) were put into the solution, with the LDH surface facing up. After 2 h, the samples were removed from the solution, washed 3 times with water, and dried at 60 °C in an oven.

Example 5: Durability

25 The Mg/Al V-LDH was adhered strongly onto the substrate which is evidenced by V-LDH on glass substrate exhibiting no obvious changes after 1 hour of ultrasonic treatment. Without being bound to theory, the V-LDH coating displays much stronger adhesion strength between the LDH coating and the glass substrate as compared to direct dip coating of free LDH particles on glass substrate, which may be due to the presence of a magnesium aluminium silicate phase at the interface between the LDH coating and the glass surface.

Example 6: Substrate Change

30 The method developed for growing V-LDH on glass substrate as disclosed herein has also been applied on other substrates, such as plastics, metal, and ceramics. Fig. 3 shows the SEM images of V-LDH grown on the surface of polycarbonate (Fig. 3a), stainless steel (Fig. 3b) and ceramic (Fig. 3c). The coatings were observed to be similar in structure to those grown on glass. Fig. 4 shows the XRD pattern of V-LDH on polycarbonate which is similar to that of V-LDH on glass (Fig. 2).

35 Example 7: Antibacterial Properties

V-LDH growing on a glass surface, which was modified by calcination at 450 °C for 18 hours as described in Example 3, displayed an average spacing between each piece of adjacent

LDH of 450 nm, which is ideal for antimicrobial application as the bacteria cells which come into contact with the surface are ruptured by the edges of LDH. Fig. 5 shows SEM images of *E. coli* (Fig. 5a), *S. aureus* (Fig. 5b) and *C. albicans* (Fig. 5c) on the modified V-LDH glass surface samples, which were ruptured and killed after incubation for 24 hours. The JIS testing results showed that only calcined V-LDH coated glass killed *E. coli* (Fig. 6a) and *S. aureus* (Fig. 6b) after a 24 hour incubation period but not as-prepared unmodified V-LDH coated glass.

Table 1 shows the effect of surface modification with oleic acid (OA), polyethylenimine (PEI) and platinum (Pt) coating on the surface hydrophilicity, surface charge and antimicrobial property. The as prepared V-LDH was positively charged. After surface modification with OA and PEI, the surface was still neutral or positively charged. However, the contact angles varied from very hydrophobic (contact angle 133.6°) after OA modification to very hydrophilic (contact angle ~0°) after PEI and Pt modification. Antimicrobial tests showed that the hydrophilic surfaces, that is those modified with PEI and Pt, are bactericidal with 7 log reduction for *E. coli* (Table 1). On the other hand, the hydrophobic surface prepared by OA modification did not show any antibacterial properties.

This same trend was also found in calcined V-LDH surfaces. Without modification, the calcined V-LDH surface was negatively charged. After surface modification with OA, PEI, Pt, L-glutamic acid and L-arginine, the surfaces remained negatively charged. However, the contact angles varied with different surface modifications. The unmodified calcined V-LDH was very hydrophilic and displayed microbicidal properties. OA modification resulted in a highly hydrophobic surface which did not display any microbicidal properties. Surface modification with PEI, Pt and L-glutamic acid provided highly hydrophilic surfaces which displayed microbicidal properties. Surface modification with another amino acid, L-arginine, led to a hydrophobic and non-microbicidal surface.

It can be seen from Table 1 that the antimicrobial effect of V-LDH is associated with surface hydrophilicity and that the higher the hydrophilicity, the better the antimicrobial property observed.

As a comparison, the surface with calcined LDH that are not vertically aligned was found to be negatively charged and hydrophilic but did not display any microbicidal properties. This shows that in addition to being hydrophilic, the surface also needs to be coated with vertically aligned LDH to display microbicidal properties.

Table 1. Effects of surface modification on surface hydrophilicity, surface charge and antimicrobial properties of V-LDH

	Modification	Charge/ mV	Contact angle / °	log reduction		
				<i>E. coli</i>	<i>S. aureus</i>	<i>C. albicans</i>
LDH-as prepared (Example 2)	No	+5.9	59.4	0	0	0
	Oleic acid (Example 4)	0.0	133.6	0	--	--
	Polyethylene imine (PEI) (Example 4)	+5.7	0	7	--	--
	Platinum (Pt) (Example 4)	-17.7	0	7	--	--
LDH- calcined (Example 3)	No	-18.9	0	7	7	2
	Without aligned structure (non-aligned LDH) (Comparative Example 1)	-18.9	0	0	0	--
	Oleic acid (Example 4)	-20.8	133.6	0	--	--
	PEI (Example 4)	-20.1	0	7	--	--
	Pt (Example 4)	-20.6	0	7	--	--
	L-glutamic acid (Example 4)	-16.2	0	7	--	--
	L-arginine (Example 4)	-17.5	71.8	0	--	--

Example 8: Implant Coating

- 5 LDH has been shown to have good blood clearance and can be well absorbed by human body. The growth of LDH on a titanium alloy implant surface was conducted and its suitability as an antimicrobial layer was assessed. The calcined V-LDH grown on a titanium surface (Fig. 7) is similar in structure as that on glass. The antimicrobial property of calcined V-LDH on titanium was evaluated using the JIS Z 2801 method against MRSA. As shown in Fig. 8, all
- 10 MRSA on calcined V-LDH coated titanium was killed after a 24 hour incubation, while MRSA on uncoated titanium continued growing after incubation. This experiment demonstrates the potential of V-LDH coating for antimicrobial implants.

Industrial Applicability

5 The disclosed substrates coated with a layered double hydroxide may be useful in a range of applications in medical device, implant and healthcare related industries. The substrate may be useful in high touch surfaces such as door handles, lift buttons, handrails, toilet fixtures, more broadly for surfaces in areas with high human traffic such as hospitals, schools, malls, public transport vehicles, airports, restaurants as well as for implanted medical devices such as stents, pacemakers, orthopaedic plates, rods, screws, catheters, hip or knee prostheses where antimicrobial properties are needed.

10 It will be apparent that various other modifications and adaptations of the invention will be apparent to the person skilled in the art after reading the foregoing disclosure without departing from the spirit and scope of the invention and it is intended that all such modifications and adaptations come within the scope of the appended claims.