

COPPER NANOPARTICLE FORMULATION TO PROMOTE RAPID PATHOGEN INACTIVATION

5 FIELD OF THE INVENTION

The present invention relates to a composite material for killing a microbe on contact, to methods of making the composite material, and to the use of the composite material.

10 BACKGROUND

Many diseases are caused by microbes, and as such there is high demand for products that provide the ability to kill microbes. For example, the spread of the SARS-CoV-2 virus has created a greater awareness for the importance of the use of protective facemasks. From
15 surgical masks to respirators, and even reusable masks from old pieces of clothing, the world population has become creative to generate vast ways to prevent the spread of respiratory infectious diseases.

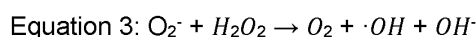
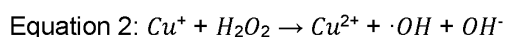
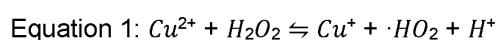
Although both organic compounds (such as phenols, chitosan) and inorganic materials (like
20 zinc, copper, silver and various metal oxides) have been demonstrated to possess biocidal properties, the latter has become the main choice in anti-bacterial formulations. Metals and their oxides can be prepared in the form of nanoparticles with high surface area-to-volume ratio which makes them highly active against pathogens. Moreover, inorganic nanoparticles also offer stability and robustness against harsh conditions when compared to their organic
25 counterparts.

Throughout human civilization, copper and its derivatives have emerged as excellent and economically viable, bactericidal candidates. The primary mechanism involves the release of electrically charged copper ions that can penetrate the pathogen membrane, destroy the
30 genetic materials and stop the genome translation. There is a consensus that firstly the copper ions damage the pathogen's envelope through the creation of holes in the membrane and by membrane peroxidation, which results in the loss of cell viability. Also, redox reaction between copper (I) and copper (II) produces reactive oxygen species (ROS) such as hydroxyl radicals that may attack lipids, proteins, DNA and other biomolecules, ultimately resulting in cell death.

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The antimicrobial properties of copper have been previously reported, and the shortest reported duration required for killing bacteria on wet surfaces is 30 minutes against *Clostridium difficile*, and more than 60 minutes against *Escherichia coli*.

- 5 The antimicrobial activity of copper through “contact-killing” is believed to be primarily due to two factors: i) the dissolution of copper ions, which can penetrate and damage the bacterial cell wall; and ii) the cyclic redox reactions between Cu^+ and Cu^{2+} , which generates reactive hydroxyl radicals (reactive oxygen species, peroxides) by Fenton-type (Equation 1 and 2) and Haber-Weiss reactions (Equation 3). These radicals may attack lipids, proteins, DNA and other
10 biomolecules resulting in microbe death.



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- While previous studies have been performed on bulk copper surfaces, the effect of variables such as oxidation state and particle size of the copper is not fully understood. However, since bulk copper will change the mechanical properties of a surface it is applied to, there is a need for antimicrobial materials that can be applied to, or formed into, soft and conformable surfaces.
20 For example, the fabric of a face mask must be non-rigid and conformable, but could beneficially be provided with antimicrobial properties. Similarly, it could also be advantageous to impart antimicrobial properties to upholstered fabric, yet coating such fabrics in bulk metal would have negative impacts on the comfort and appearance of the fabric. Therefore, while it is important to be able to impart antibacterial properties to such flexible and breathable
25 materials in order to safeguard the population against infectious pathogens, many such surfaces cannot be coated with bulk copper without negatively affecting the mechanical, comfort and aesthetic properties of the surface. There is therefore a need for the ability to impart long term antimicrobial properties to such fabrics (e.g. in face masks) without degrading the conformability and appearance of the fabric.

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- Various woven and non-woven materials have been used to construct facemasks, such as household fabrics, blends of natural and synthetic fibers, and solely synthetic fibers. There are multiple factors influencing the material selection, including availability, cost, comfort, breathability, quick-dry ability, etc. However, as facemasks are intended as personal
35 protection equipment, the particle filtration properties of the fabrics are of utmost importance. The efficacy of a facemask is determined by the percentage of droplets percolated through the filter material, and the pressure drop assessed by the air resistance across the material

that directly impacts breathability. The combination of filtration efficiency and pressure drop allows the derivation of the filtration quality factor, Q . Therefore, maximum Q should arise from low droplet penetration with low pressure drop. In addition to the physical/mechanical barrier provided by the fabrics, the other means of protection is frequently provided *via* triboelectric charging of the fibers, which acts by trapping particles *via* electrostatic interactions, thus improving the filtration efficacy without compromising the breathability. Another strategy to improve the filtration efficiency is by employing multilayered filters.

The variation in the filtration efficiency is often attributed to the construction of the filter material and the thread count number. A triboelectric treatment can also induce different effects depending on the type of fabrics used. Generally, there is little change in the filtration efficiency and quality factor Q for cotton upon charging, while the properties of polyester, nylon, silk and polypropylene can be significantly improved. Synthetic fabrics such as polypropylene, polyester or polyurethane are excellent candidates for triboelectric charging, yet this effect is strongly decreased with ambient humidity. Therefore, research suggests that there is a complex interplay between fabric material, weave and yarn counts, and filtration efficiency.

A major limitation in the effectiveness of current face masks is that while they may prevent the transmission of pathogens through the mask, there will be a build-up of pathogens on and within the mask, which could lead to transmission of diseases if the mask is handled improperly during or after use. In addition, masks typically prevent transmission of pathogens by utilising the triboelectric effect to provide highly electrostatically charged filter materials that block a high proportion of airborne droplets. For example, N95 respirators are designed to fit tightly around the nose and mouth, which makes them more effective in stopping the spread of infectious airborne particles. Additionally, the filter material in respirators has a higher electrostatic charge that blocks 95% of airborne droplets with a diameter as small as 0.3 micrometers. However, the triboelectric effect may be compromised in high humidity environments, making these masks potentially less effective in tropical countries.

Existing technologies focus on triboelectric effect to achieve high filtration quality while ignoring the pathogen accumulation on the fabrics. Some technologies may incorporate the nanoparticles directly into the bulk fabrics, and not necessarily limited to the surface.

There is therefore a need for materials that are able to block the transmission of pathogens/microbes in high humidity environments and also inactivate these pathogens/microbes on contact. In other words, there is an urgent need to develop cost-effective coatings that can be applied to readily available materials to impart multiple

functionalities, including contact-killing of bacteria and viruses within the shortest time possible (i.e. less than 30 minutes).

5 Nanoparticles (NPs) have a high surface-area-to-volume ratio that makes them highly reactive and they are potential candidates to endow surfaces with higher antibacterial activity as compared to solid bulk copper surfaces. Recently, researchers have investigated the stress-induced rupture of the bacterial cell membrane, especially for large non-translocating NPs. They demonstrated that the adsorption of gold NPs on the surface of bacteria increases the membrane tension, hence causing membrane deformation and rupture. However, the effect
10 of metallic copper or copper NPs as an antibacterial agent is not known.

SUMMARY OF THE INVENTION

15 The inventors have surprisingly found that a composite material comprising nanoparticles that comprise Cu₂O adhered to the surface of a substrate provides sustained, rapid and effective killing of microbes. The incorporation of Cu₂O nanoparticles into a filter material (such as a fibrous material in a face mask) may therefore, at least in part, solve the problems discussed above. In other words, the invention disclosed herein can activate an initially non-active surface using nanoparticles, while conventional technologies make use of active bulk
20 materials to activate the surface.

Aspects and embodiments of the invention will now be referred to by the following numbered clauses.

- 25 1. A composite material for killing a microbe on contact comprising:
a substrate material having a surface;
a silicon-containing material attached to the surface of the substrate material; and
a plurality of nanoparticles comprising Cu₂O attached to the silicon-containing material.
- 30 2. The composite material according to Clause 1, wherein the substrate material is selected from one or more of a glass, a polymer, and a fibrous material.
3. The composite material according to Clause 2, wherein:
(aa) the fibrous material is selected from one or more of the group consisting of cotton,
35 linen, silk, polyester (such as woven- or non-woven polyester), polypropylene (such as a meltblown polypropylene or spun bond polypropylene), polyurethane, cellulose,

cellulose/polyester blend, rayon, optionally wherein the fibrous material is in the form of a woven or non-woven fabric; and/or

(bb) the fibrous material comprises a plurality of fibres and the silicon-containing material coats at least part of some of the plurality of fibres.

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4. The composite material according to any one of the preceding clauses, wherein the silicon-containing material comprises a silicon-containing polymer or a silicon-containing small molecule, optionally wherein the silicon-containing polymer comprises a siloxane polymer.

10 5. The composite material according to Clause 4, wherein:

(AA) the silicon-containing small molecule is selected from one or more of tetraethylorthosilicate (TEOS), aminoethylaminopropyltrimethoxysilane (AEAPTMS), 3-aminopropyltriethoxysilane (APTES), methacryloyloxypropyltrimethoxysilane (MPTMS), hexadecyltrimethoxysilane (HDTMS), n-octadecyltriethoxysiloxane (ODTMS); or

15 (BB) the silicon-containing polymer is polydimethylsiloxane (PDMS).

6. The composite material according to any one of the preceding clauses, wherein the silicon-containing material has a water contact angle of from 130° to 150°, optionally wherein the silicon-containing material has a water contact angle of from 134° to 145°, such as from
20 134.5° to 140°.

7. The composite material according to any one of the preceding clauses, wherein the composite material is porous.

25 8. The composite material according to any one of the preceding clauses, wherein the composite material is washable.

9. The composite material according to Clause 8, wherein after being subjected to a 6-hour wash cycle in an aqueous solution of Triton-X 100 (2-[4-(2,4,4-trimethylpentan-2-yl)phenoxy]ethanol) (1 g/L) in ultrapure water (18 mΩ) at a liquor ratio of 50:1 ml/g in an ultrasound bath (275 W) at 45°C, the composite material:

30 (ia) retains at least 90 mol %, such as at least 95 mol %, e.g. at least 99 mol % of the original amount of copper (I) oxide; and/or

(ib) retains a water contact angle of $\pm 10^\circ$, such as $\pm 5^\circ$, $\pm 2^\circ$, e.g. $\pm 1.5^\circ$, as compared
35 to the water contact angle of the composite material before washing.

10. The composite material according to any one of the preceding clauses, wherein less than 20% by weight of the initial loading of the nanoparticles comprising Cu_2O is lost from the composite material after being subjected to a 6-hour wash cycle in an aqueous solution of Triton-X 100 (2-[4-(2,4,4-trimethylpentan-2-yl)phenoxy]ethanol) (1 g/L) in ultrapure water (18 mΩ) at a liquor ratio of 50:1 ml/g in an ultrasound bath (275 W) at 45°C, optionally wherein less than 15%, less than 10%, less than 5%, less than 4%, less than 3%, less than 2%, or less than 1% by weight of the initial loading of the nanoparticles comprising Cu_2O is lost from the composite material after being subjected to a 6-hour wash cycle as described above.
11. The composite material according to any one of the preceding clauses, wherein:
- (ai) the nanoparticles comprising Cu_2O have a mean diameter of from 10 to 500 nm, optionally from 20 to 300 nm, more optionally from 30 to 200 nm, for example from 40 to 150 nm, such as about 73.5 nm; and/or
- (aii) the nanoparticles comprising Cu_2O form from 2 to 15 wt% of the total weight of the composite material, optionally wherein the nanoparticles comprising Cu_2O form from 3 to 13 wt%, such as from 4 to 11 wt%, such as about 4.9 wt% or about 9.5 wt%, of the total weight of the composite material.
12. A method of making a composite material according to any one of Clauses 1 to 11, comprising the steps of:
- (i) providing an intermediate material formed from:
- a substrate material having a surface; and
- a silicon-containing material attached to the surface of the substrate material;
- and
- (ii) attaching a plurality of nanoparticles comprising Cu_2O onto the silicon-containing material.
13. The method according to Clause 12, wherein the intermediate material is formed by:
- (bi) providing a substrate material having a surface; and
- (bii) attaching a silicon-containing material onto the surface of the substrate material, optionally wherein step (bii) comprises a preliminary step of activating the surface of the substrate material, such as by plasma surface activation.
14. The method according to Clause 12 or Clause 13, wherein step (ii) of Clause 12 is performed by dip-coating of the intermediate material into a dispersion of nanoparticles comprising Cu_2O .

15. The method according to Clause 12 or Clause 13, wherein step (ii) of Clause 12 is performed by spray-coating a dispersion of nanoparticles comprising Cu₂O onto the intermediate material,

optionally wherein the atomic concentration of copper after spray-coating is from 5 to 15% as analysed by SEM-EDX maps at 500x magnification.

16. The method according to Clause 14 or Clause 15, wherein the dispersion of nanoparticles comprising Cu₂O is a dispersion of Cu₂O-containing nanoparticles in an organic solvent, optionally wherein the organic solvent is an alcohol (e.g. the organic solvent is selected from one or more of the group consisting of methanol, ethanol, and isopropyl alcohol).

17. The method according to Clause 16, wherein the ratio of nanoparticles comprising Cu₂O to organic solvent is from 0.5 to 5 mg nanoparticle/mL of solvent, such as from 0.7 to 2 mg nanoparticle/mL of solvent, such as about 1 mg nanoparticle/mL of solvent.

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18. Use of a composite material as described in any one of Clauses 1 to 11 as an antimicrobial surface.

BRIEF DESCRIPTION OF THE FIGURES

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Figure 1. (a) The fabrication route and characterization of the copper coatings. The copper nanoparticles were characterized using SEM-EDS, XPS, TEM-EELS and AES and dispersed with the aid of an ultrasonic bath, followed by spray-coating on the substrates. Subsequently, the copper-coated fabrics were inoculated with Gram-positive and Gram-negative bacteria and SEM images were collected. The amount of oxidative damage and bacterial genomic DNA fragmentation after bacterial interaction was assessed. The images (b-f) correspond to yCu (Cu₂O), while the images (g-k) correspond to aCu. (b, g) SEM and (c, h) STEM images, (d, i) XPS Cu 2p core level spectrum, (e, j) AES Cu LMM spectrum, (f, k) TEMEELS Cu L_{2,3} edges.

Figure 2. Distribution of the nanoparticle aggregates on the fabrics after the spray-coating process. SEM images and EDS Cu K α maps of yCu (Cu₂O) coated on (a-b) Fabric #1 and on (e-f) Fabric #2. SEM images and EDS Cu K α maps of aCu (metallic copper) coated on (c-d) Fabric #1 and (g-h) Fabric #2. The scale bar is 10 μ m.

Figure 3. yCu (Cu₂O) and aCu (metallic copper) fabrics exhibit antibacterial properties. (a) Schematic illustration of the bacterial wall damage generated by copper nanoparticles in contact with bacteria. (b-d) SEM images of the fabrics after inoculation with *Klebsiella*

pneumoniae ENT646. (e-g) Bacterial counts obtained after various fabric interactions with (e) carbapenem-resistant hypervirulent *Klebsiella pneumoniae* ENT646, (f) *Pseudomonas aeruginosa* PAO1 and (g) *Staphylococcus aureus* SA29213. Circular fabrics of 6 mm diameter were inoculated with 10^4 CFU bacteria for a duration of 45 seconds before the enumeration of bacterial counts. (h-i) To examine the robustness of the fabrics, 10^4 CFU of *Klebsiella pneumoniae* ENT646 was added to (h) F#1 + yCu (Cu_2O) or (i) F#1 + aCu (metallic copper) fabrics cumulatively at the indicated timepoints. Bacterial counts were examined at every timepoint. (j) Bacterial genomic DNA was assessed for DNA fragmentation after bacterial interaction with the indicated fabrics. Circular fabrics of 6 mm diameter were inoculated with 10^8 CFU bacteria for a duration of 45 seconds or 1 hour before extraction of DNA and examination of DNA fragmentation via gel electrophoresis. (k) Amount of oxidative damage marker 8-hydroxy-2'-deoxyguanosine (8OHdG) after DNA interaction with the fabrics. 50 μg of DNA was added to circular fabrics of 6 mm diameter for 45 seconds or 1 hour before DNA retrieval and examination via LC-MS. Levels of 8OHdG are expressed relative to 10,000dG. All the assays were performed in triplicates and the values are expressed as mean $\pm$ S.D.

Figure 4. SEM images and EDS maps of the fabrics coated with copper nanoparticle aggregates at low magnification, 100x. Fabric #1 coated with (a-c) yCu (Cu_2O) and (d-f) aCu. Fabric #2 coated with (g-i) yCu (Cu_2O) and (j-l) aCu. The left panel corresponds to the SEM images, in the middle are the EDS maps and EDS spectra from the maps with quantifications are in the right panel. The scale bar corresponds to 100 μm .

Figure 5. XPS Cu 2p core-level spectrum corresponding to old yCu (Cu_2O) sample. The sample has been left in ambient conditions for at least a month. The peak-fitting uses Gaussian–Lorentzian (GL) line shapes using FWHM, spectral component separation (eV) and peak areas based on published literature.

Figure 6. XRD patterns of aCu (metallic copper) and yCu (Cu_2O).

Figure 7. Survey-scan AES spectra of aCu (metallic copper) and yCu (Cu_2O).

Figure 8. (a-b) Bacterial counts obtained after various fabric interactions with carbapenem-resistant hypervirulent *Klebsiella pneumoniae* ENT646. Circular fabrics of 6 mm diameter were inoculated with (a) 10^5 CFU bacteria or (b) 10^6 CFU bacteria for a duration of 45 seconds before the enumeration of bacterial counts. Bacterial numbers obtained from fabrics with yCu (Cu_2O) or aCu (metallic copper) were compared to the average bacterial counts obtained from control fabrics F#1 or F#2 to obtain percentage killing according to the formula: $100 - (\text{counts})$

from the fabric of interest/average counts from control*100). Percentage killing values are expressed in a table within each subfigure. The assays were performed in triplicates and the values are expressed as mean $\pm$ S.D. (c) Bacterial counts obtained after various fabric interactions with carbapenem-resistant hypervirulent *Klebsiella pneumoniae* ENT646. Circular fabrics of 6 mm diameter were inoculated with 10^8 CFU bacteria for a duration of 45 seconds or 1 hour before the enumeration of bacterial counts. The assay was performed in duplicates. Mean of the datapoints is shown in the graph as a line. Percentage killing was calculated by comparing the mean bacterial load obtained from F#1 + yCu (Cu_2O) or F#1 + aCu (metallic copper) and compared to the mean bacterial counts from control F#1 and is expressed in a table within the subfigure. (d) Bacterial counts obtained after various fabric interactions with *Staphylococcus aureus* SA29213. Circular fabrics of 6 mm diameter were inoculated with 10^4 CFU bacteria for a duration of 45 seconds before the enumeration of bacterial counts. The assay was performed in duplicates. Mean of the datapoints is shown in the graph as a line.

Figure 9. XPS spectra from yCu- (Cu_2O) and aCu-coated fabrics before and after addition of deionized water. The spectra correspond to three time points: dried fabric with no water (no- H_2O), after the first addition of water ($\text{H}_2\text{O}_{\text{initial}}$), and after the last addition of water at $t=144$ hours ($\text{H}_2\text{O}_{\text{end}}$).

Figure 10. Performance of the fabric coated with the formulation (a) before washing and (b) after washing in an aqueous solution of Triton X-100 at 45 °C for six hours. (1) SEM images, (2) EDX maps, (3) contact angle images of the fabrics. (c) FTIR-ATR spectra of the fabrics.

Figure 11. Meltblown polypropylene filter coated with the copper formulation. (a) Filter without formulation, (b) dip-coated filter, (c) spray-coated filter. Series 1, 2 and 3 correspond to optical photographs of the filters, scanning electron micrographs, and energy-dispersive X-ray maps, respectively. (a3) shows the carbon element, and (b3 and c3) show the copper element. Scalebar = 50 μm .

Figure 12. An SEM image of fabric fibres coated with nanoparticles (dip coating).

Figure 13. Antibacterial tests conducted on various copper NP coatings on cotton substrate. (a-b) Bacterial counts obtained after various fabric interactions with carbapenem-resistant hypervirulent *Klebsiella pneumoniae* ENT646. Circular fabrics of 6 mm diameter were inoculated with 10^4 CFU bacteria for a duration of 30 minutes before the enumeration of bacterial counts. Samples labelled from A to F correspond to pristine cotton, CuO-MPTMS-coated cotton, Cu₂O-HDTMS-coated cotton, CuO-coated cotton, Cu₂O-coated cotton (NP size

around 100 nm), and Cu₂O-coated cotton (NP size around 7 microns), respectively. (c) Antibacterial report for sample E (Cu₂O-coated cotton) using the standard GB/T 21866-2008.

Figure 14. Various fabric materials dip-coated with the formulations of two different copper nanoparticles (NP1 and NP2). (a, b) are woven cotton fabrics; (c, d) are woven polyester (PET) fabrics; (e, f) are non-woven spunbond polypropylene (PP) fabrics; (g, h) are nonwoven melt-blown PP fabrics; and (i, j) are non-woven polyester fabrics.

DETAILED DESCRIPTION

The invention provides a composite material for killing a microbe on contact comprising:

a substrate material having a surface;

a silicon-containing material attached to the surface of the substrate material; and

a plurality of nanoparticles comprising Cu₂O attached to the silicon-containing material.

Existing facemask technology focuses on the triboelectric effect to trap water droplets as well as physically filtering the particles. It has been surprisingly found that microbes can be rapidly killed on a surface that has been treated with (or is formed from) the composite material of the current invention. Applications for the composite materials disclosed herein include, but are not limited to the following applications.

A. Applying reactive copper nanoparticles on woven and non-woven fabrics, which when in contact with pathogen-loaded water droplets can kill, prevent, or even stop the chain of infection caused by pathogenic agents. The additional fabric features will further promote contact between the pathogens and copper ions, which may enhance the killing and inactivation of the pathogens. This innovation is applicable on a range of substrates, from household materials to upholstery fabrics, with very little change needed in the processing of various substrate materials, making this a very versatile technology.

B. Intensifying the exposure of reactive copper ions on the surfaces for quick pathogen-killing action. The current innovation is primarily based on the use of cuprous oxide (Cu₂O) nanoparticles, which are shown herein to be the most effective form of copper derivative in killing bacteria compared to other copper species. The reduction in the killing time may be shortened by increasing the nanoparticle coverage on the fabrics. Furthermore, the biocidal efficacy may also be improved through mask design, which directs the droplets to “killing” sites.

The composite materials disclosed herein have two distinctive features, namely surface compatibility (of the copper nanoparticles with the substrate material that they are attached to via the silicon-containing material) and enhanced reactivity, unlike commercially available solutions. The composites disclosed herein work regardless of the substrate (e.g. fabric), structure (e.g. woven and non-woven in the case of a fabric) and the type of substrate material (e.g. in the case of a fabric, its natural and/or synthetic origin). In the case of a fabric, this may enable one to exploit the fabrics' inherent filtration efficiencies. As will be appreciated, the methodology disclosed hereinbelow also allows for a composite material to be formed on existing fabrics as a post-treatment process.

As used herein, killing a microbe (e.g. a bacterium or a fungus) on contact means that the composite material is able to kill at least 80%, such as at least 90%, at least 95%, at least 99%, at least 99.9%, or more preferably, at least 99.99% of microbes within 45 seconds when 5 μ L of an aqueous microbial suspension comprising 10⁵ CFU is applied to a circular sample of the composite material having a diameter of 6 mm. The same ratios may apply to viruses, where a similar test may be conducted. For example, the test may use 5 μ L of an aqueous viral suspension comprising 10⁵ PFU applied to a circular sample of the composite material having a diameter of 6 mm.

The term "microbes" when used herein may apply to any microbial species, which includes, but is not limited to bacteria, viruses and fungi. Particular embodiments of the invention may relate to a composite material that is suited to killing bacteria and/or viruses.

The composite material includes a substrate material, also referred to as simply "substrate", which may be any material onto which it is desirable to provide an antimicrobial coating/impart antimicrobial properties. For example, the substrate may be selected from the group including, but not limited to, a glass, a polymer and a fibrous material, and combinations thereof. Other materials that may be mentioned include a metal, which may be used in combination with any of the other materials mentioned herein. In some embodiments of the invention that may be mentioned herein, the substrate may comprise a fibrous material. Examples of suitable fibrous substrates that may be mentioned herein include fabrics. Specific examples of suitable fibrous substrates that may be mentioned herein include one or more of the group consisting of cotton, linen, silk, polyester (such as woven- or non-woven polyester), polypropylene (such as a meltblown polypropylene or spun bond polypropylene), polyurethane, cellulose, cellulose/polyester blend, and rayon. The fibrous material may be in the form of a woven or non-woven fabric.

Any suitable fiber may be used. This may be a natural fiber (e.g., including but not limited to cotton, linen, silk) or a synthetic fiber (e.g., including but not limited to polypropylene, polyester, and polyurethane). As will be appreciated, any suitable combination of fibers (e.g., two, three or four natural; two, three or four synthetic; or one or two natural and one or two synthetic fibers may be used in combination as a blended fibre).

The substrate, and in particular, the fabrics may also have a hydrophilic or a hydrophobic surface, which can widen the range of applications. The hydrophilicity or hydrophobicity of the surface may be natural to the material or it may be the result of a treatment applied to the material, as known by a person skilled in the art of fabric treatment.

Based on the measured water contact angle (WCA) values, a substrate may be defined as super-hydrophilic, hydrophilic, hydrophobic, or super-hydrophobic. The contact angle of a drop of water on a solid, planar substrate may be measured directly on a drop resting on a horizontal plane (a sessile drop) captured at a solid-liquid interface. Herein, a surface with:

WCA $< 5^\circ$ is considered to be super-hydrophilic;

$5^\circ < \text{WCA} < 90^\circ$ is considered to be hydrophilic;

$90^\circ < \text{WCA} < 150^\circ$ is considered to be hydrophobic; and

WCA $> 150^\circ$ is considered to be super-hydrophobic.

The use of a hydrophobic substrate may prevent an excessive amount of pathogen-loaded droplets of water from sitting on the substrate (the first layer of protection). However, a super-hydrophobic substrate may not allow enough time for the antibacterial coating to interact with the pathogen, which may potentially reduce the antibacterial effectiveness of the substrate. Therefore, for substrates that are used, they may be hydrophobic, but are not super-hydrophobic.

When the substrate is a fabric, it may be in any suitable form. For example, the composite material may be used on an extensive range of fabrics for household applications such as upholstery, bedding, curtains, etc. and on surfaces where a low cost and contact-killing biocidal surface is needed. This approach may be particularly applicable to fabric materials used to fabricate facemasks due to the significant biocidal properties of fabric composite materials described herein. This is because it may take less than 30 minutes (e.g. less than a minute) upon contact of the microbe with the surface of the composite material for the microbe to be inactivated/killed, thereby reducing risk to a wearer (or to others in contact with the wearer if the latter is infected) during use of or upon disposal of the facemask. The copper oxide nanoparticles are bound to the surface of the fabrics by chemical bonds that can resist

extensive washing, which can improve the composite material's durability, making it suitable for not only disposable masks but also reusable masks.

When the substrate comprises a fibrous material, the silicon-containing material may be
5 attached to the individual fibres of the fibrous material, for example such that the silicon-
containing material does not fill the voids between the fibres of the fibrous material. Thus, in
some embodiments of the invention, the silicon-containing material may form a coating around
at least part of some of the individual fibres of the fibrous material. Since, in some
embodiments of the invention, the silicon-containing material may not fill the voids between
10 the fibres of the fibrous material, the porosity (and breathability) of the fibrous material may
remain substantially unchanged. In other words, the silicon-containing material may coat the
individual fibres, and no film is created. Therefore, the breathability (of the substrate material)
is not affected. This is clearly demonstrated by the SEM images discussed in the examples,
which shows the nanoparticles sitting on the fibres without a continuous film formation.

15 In some embodiments that may be mentioned herein, the substrate material may be a porous
material (e.g. a porous fabric-like material, a porous glass (e.g. a sintered glass frit), a metal
mesh, a porous polymeric material). In other embodiments, the substrate material may be a
non-porous material (e.g. a non-porous glass, metal or polymeric surface, and the like). When
20 the substrate material is a porous substrate material, the composite material may have a
porosity that is substantially unchanged compared to that of the substrate material itself. For
example, the composite material may have a porosity of at least 70% of that of the substrate
material, such as at least 80%, at least 90%, at least 95% or at least 99%. Advantageously,
maintaining the porosity of the substrate can ensure that when the composite material is used
25 in a face mask, the pressure drop across the face mask is not overly high, which is essential
to ensure that a wearer's breathing is not overly inhibited.

As noted hereinbefore, the composite material comprises a silicon-containing material that is
attached to the surface of the substrate material. The silicon-containing material may be a
30 silicon-containing polymer or a silicon-containing small molecule (e.g. a silicon-containing
molecule with a molecular weight of less than 900 Daltons, such as less than 500 Daltons). In
embodiments of the invention when the silicon-containing material is a silicon-containing small
molecule, it may be selected from one or more of tetraethylorthosilicate (TEOS),
aminoethylaminopropyltrimethoxysilane (AEAPTMS), 3-aminopropyltriethoxysilane (APTES),
35 methacryloyloxypropyltrimethoxysilane (MPTMS), hexadecyltrimethoxysilane (HDTMS), n-
octadecyltriethoxysiloxane (ODTMS). In some embodiments of the invention that may be
mentioned herein, the silicon-containing material may comprise a silicon-containing polymer.

Examples of silicon-containing polymers include, but are not limited to siloxane polymers. Siloxane polymers that may be mentioned herein include, but is not limited to polydimethylsiloxane (PDMS). For the avoidance of doubt, PDMS may be used in combination with one or more of the other siloxane polymers. In particular embodiments that may be
5 mentioned herein, the siloxane polymer may be PDMS.

An adhesive may also be applied as a top-coating to the composite material. That is, after the composite material has been formed, an adhesive coating may be applied to the composite material by spray coating or by dip-coating. Without wishing to be bound by theory, it is
10 believed that the adhesive may help to prevent significant loss of the plurality of nanoparticles comprising Cu_2O from the composite material (e.g. during washing of the composite material). Any suitable adhesive may be used for this purpose, such as an adhesive formed from an acrylic polymeric blend (e.g. the adhesive may be 3M 2262 from 3M, Minnesota, US).

As noted above, the silicon-containing material may function as a linker material between the substrate and the Cu_2O -containing nanoparticles. This may provide stronger attachment of the Cu_2O -nanoparticles to the substrate. The linker may also provide beneficial properties, such as hydrophobicity. A hydrophobic composite material may help to minimize wetting and hence absorption of pathogen-loaded polar medium or matter onto the substrate (e.g. droplets
20 comprising bacteria or viruses). However, if the composite material is too hydrophobic (e.g. superhydrophobic) then this may prevent sufficient contact between the microbes and the surface of the composite material, preventing effective killing of microbes that do settle onto the composite material. As such, it is advantageous for the composite to be hydrophobic but not necessarily superhydrophobic. Therefore, in some embodiments of the invention, the
25 silicon-containing material may have a water contact angle of from 130° to 150° , such as from 134° to 145° , e.g. from 134.5° to 140° .

The silicon-containing material may be attached to the substrate in any appropriate way. Similarly, the plurality of nanoparticles comprising Cu_2O may be attached to the silicon-containing material in any appropriate way. When a component is described herein as being
30 attached to another component, the attaching may be covalent (e.g. attached by covalent bonds between the two components) or non-covalent (e.g. attached by ionic/electrostatic interactions, or by non-ionic/electrostatic interactions, such as Van der Waal's interactions). The attachment/bonding may also be a combination of these types. Without wishing to be
35 bound by theory, it is believed that the silicon-containing material primarily forms covalent bonds with the substrate surface to which it is attached.

The composite material comprises a plurality of nanoparticles comprising Cu₂O attached to the silicon-containing material. The use of Cu₂O nanoparticles provides an advantageously increased antimicrobial effect as compared to using CuO or metallic copper. It is noted that although the bulk oxide material is stable, the surface of Cu₂O can oxidise and form CuO when
5 exposed to ambient humidity (e.g. see Camacho-Espinosa *et al. Journal of Applied Physics*, **123**, 085301 (2018)). The Cu₂O phase in the composite material undergoes minimum change and can even survive machine washing for extended periods of time. In the current invention it was noted, surprisingly, that the Cu₂O surface only oxidised to a hydroxylated Cu(OH)₂ phase that was either still active or which could be removed upon heating the
10 composite material to 100 °C. In any event, it is believed that the primary killing mechanism is still provided by the presence of Cu₂O.

In addition, the use of nanoparticles in the composite material of the invention may help provide an increased microbial killing effect compared to the use of microparticles or bulk
15 copper oxide surfaces. Without wishing to be bound by theory, this is believed to be due to the increased active surface area provided by nanoparticles compared against that provided by the same weight of microparticles or bulk material. In some embodiments of the invention that may be mentioned herein, the nanoparticles comprising Cu₂O may have a mean diameter of from 10 to 500 nm, such as from 20 to 300 nm, from 30 to 200 nm, such as from from 40 to
20 150 nm, such as about 73.5 nm. The degree of variation within the listed ranges may be from ±0.1 nm to ±5 nm, such as from ±0.5 nm to ±2.5 nm, such as from ±0.75 nm to ±2.2 nm. The size (diameter) of the particles may be calculated from scanning electron micrographs collected from pristine particles. More particularly, the mean diameter may be calculated based on the mean value of at least 100 particles from an SEM image at 100,000x
25 magnification. In some embodiments of the invention that may be mentioned herein, the nanoparticles comprising Cu₂O may form from 2 to 15 wt% of the total weight of the composite material, for example from 3 to 13 wt%, such as from 4 to 11 wt%, e.g. about 4.9 wt% or about 9.5 wt%, of the total weight of the composite material.

30 In some embodiments of the invention that may be mentioned herein, the composite material may be washable. For example, the composite material may be substantially unchanged after a 6-hour wash cycle with Triton-X 100 (2-[4-(2,4,4-trimethylpentan-2-yl)phenoxy]ethanol) at 45°C, where the wash cycle may comprise adding the composite material into an aqueous solution of Triton-X 100 (1 g/L) in ultrapure water (18 mΩ) at a liquor ratio of 50:1 ml/g and
35 subjecting the composite material to washing in an ultrasound bath (275 W) at 45 °C. The composite material may be subjected to washing for 6 hours (e.g. performed as approximately 120 washing cycles (3 min/cycle).

In some embodiments of the invention that may be mentioned herein, after washing under the above conditions, the composite material may have one or more of the following properties:

- (ia) retain at least 90 mol %, such as at least 95 mol %, e.g. at least 99 mol % of the original amount of copper (I) oxide (in other words, at least 90 mol % of the copper (I) oxide present in the composite material remains after washing and is not lost from the composite material or oxidised to copper (II) oxide);
- (ib) retain a water contact angle of $\pm 10^\circ$, such as $\pm 5^\circ$, $\pm 2^\circ$, e.g. $\pm 1.5^\circ$, as compared to the water contact angle of the composite material before washing; and
- (ic) lose less than 20% by weight of the initial loading of the nanoparticles comprising Cu_2O from the composite material (for example less than 15%, less than 10%, less than 5%, less than 4%, less than 3%, less than 2%, or less than 1% by weight) (in other words, less than 20% by weight of the nanoparticles are lost during the washing process).

15

For the avoidance of doubt, property (ia) refers to the ability of each nanoparticle to retain a certain amount of the original amount of copper (I) oxide even after the washing process (whether or not it remains part of the composite material), while property (ic) refers to how much of the original loading of nanoparticles is retained in the composite material after it has been washed.

20

The stability of the composite material to washing in an aqueous environment as described above and demonstrated in the below Examples also shows the stability of the composite material in a high-humidity environment, and suggests that the composite material will be highly stable for long periods of time in humid environments.

25

The invention also provides a method of making the composite material. The method may comprise the steps of:

- (i) providing an intermediate material formed from:
 - a substrate material having a surface; and
 - a silicon-containing material attached to the surface of the substrate material;
 and
- (ii) attaching a plurality of nanoparticles comprising Cu_2O onto the silicon-containing material.

30

The plurality of nanoparticles comprising Cu_2O may be attached to the silicon-containing material *via* any appropriate method, such as spray coating or dip coating. In some

embodiments of the invention that may be mentioned herein, step (ii) above may be performed by dip-coating of the intermediate material into a dispersion of nanoparticles comprising Cu_2O , or step (ii) above may be performed by spray coating a dispersion of nanoparticles comprising Cu_2O onto the intermediate material. Other methods of providing the coating that may be mentioned herein include, but are not limited to roll-to-roll coating. This method may allow customized patterned impregnation of the nanoparticle formulation onto the surface of the intermediate material (e.g. one comprising fabrics).

In some embodiments of the invention, the plurality of nanoparticles comprising Cu_2O may be attached to the silicon-containing material by spray coating a dispersion of nanoparticles comprising Cu_2O onto the intermediate material. Spray coating may be advantageous because it does not involve a long drying time for evaporation of solvent and may involve smaller amounts of chemicals than dip coating. In addition, spray coating may be performed on existing products for which dip coating would not be practical – such as bulky upholstered fabric. Spray coating may also be formulated to use a minimum amount of solvent, which provides cost, efficiency and environmental benefits.

Furthermore, spray coating may easily be used to provide multiple coats of the nanoparticles as required. As such, spray coating may be used to provide composite materials having higher amounts of copper more conveniently than using dip coating. Thus, in some embodiments of the invention, the atomic concentration of copper after spray-coating may be from 2 to 15 wt% of the total weight of the composite material, for example from 3 to 13 wt%, such as from 4 to 11 wt%, e.g. about 4.9 wt% or about 9.5 wt%, as analysed by SEM-EDX maps at 500x magnification. This may advantageously provide an improved anti-microbial effect as compared to composite materials having lower amounts of copper.

Both spray coating and dip coating involve the use of a dispersion of nanoparticles comprising Cu_2O . Such a dispersion may be a dispersion in an organic solvent, for example an alcohol. In some embodiments of the invention that may be mentioned herein, the organic solvent may be selected from one or more of the group consisting of methanol, ethanol, and isopropyl alcohol.

The ratio of nanoparticles comprising Cu_2O to organic solvent in the dispersion may be from 0.5 to 5 mg nanoparticle/mL of solvent, such as from 0.7 to 2 mg nanoparticle/mL of solvent, such as about 1 mg nanoparticle/mL of solvent.

The intermediate material used in the method disclosed above may be formed by:

- (bi) providing a substrate material having a surface; and
- (bii) attaching a silicon-containing material onto the surface of the substrate material.

In some embodiments of the invention, it may be advantageous to perform a surface activation step on the substrate material in order to ensure sufficient adhesion of the silicon-containing material. An example of a suitable activation process is the use of a plasma. More particularly, if the substrate material is formed from or comprises an alkene-based polymer (e.g. one or more of a polypropylene, a polyethylene (of any density, e.g. HDPE) and a fluoropolymer), a plasma surface activation step may be performed to improve adhesion of the silicon-containing material, though a skilled person will understand that a surface activation step (e.g. a plasma surface activation step) may also be useful for other substrates. Thus, in some embodiments of the invention step (bii) comprises a preliminary step of activating the surface of the substrate material, such as by plasma surface activation.

15 EXAMPLES

General Materials and Methods

Chemicals and fabrics: yCu (Cu_2O) was purchased from Chongqing Yumeco Import & Export Co., Ltd. (China). aCu (metallic copper) was supplied by Kuprion Inc. (USA). A commercial plastic adhesive (3M 2262) was purchased from 3M (USA). Sylgard 184 (polydimethylsiloxane, PDMS) was purchased from Dow (Singapore). Tetraethylorthosilicate (TEOS) aminoethylaminopropyltrimethoxysilane (AEAPTMS), 3-aminopropyltriethoxysilane (APTES), methacryloyloxypropyltrimethoxysilane (MPTMS), hexadecyltrimethoxysilane (HDTMS), and n-octadecyltriethoxysiloxane (ODTMS) were purchased from Sigma (Singapore). Acetone and isopropyl alcohol (IPA) were purchased from Aik Moh (Singapore). Tetrahydrofuran (THF) was purchased from Tedia (Singapore). These compounds were used as received.

Fabric #1 was a 55/45% cellulose/polyester fabric blend, Fabric #2 was a 70/30% rayon/polyester fabric blend, and Fabric #3 was a glossy vinyl sheet (AIVA) kindly supplied by Kuprion.

aCu (1% w/w) was dispersed in IPA and sonicated in ultrasonic bath for 2 minutes. The same method was employed for the yCu powder. The plastic adhesive (2.5% w/w) was dissolved in a mixture of acetone and IPA at a 1:4.5 weight ratio prior to its use.

General Methods Suitable for Coating of the Substrate with Silicon-containing Material:

To activate the surface of alkene-based polymer substrates (e.g., PP), the substrate was subjected to surface plasma treatment before coating with the silicon-containing material. The alkene-based substrate was treated in a plasma environment with 30 standard cubic centimetres per minute (sccm) of O₂ and 30 sccm Ar at 40 W under a base purge of 5.00 e⁻² Torr for 5 minutes.

To ensure compatibility between the copper nanoparticles and the substrate, two routes were investigated. In the first approach, the nanoparticles were functionalised with coupling agents, such as tetraethylorthosilicate (TEOS) aminoethylaminopropyltrimethoxysilane (AEAPTMS), 3-aminopropyltriethoxysilane (APTES), methacryloyloxypropyltrimethoxysilane (MPTMS), hexadecyltrimethoxysilane (HDTMS), and n-octadecyltriethoxysiloxane (ODTMS). The silane linker was first hydrolysed in ethanol (1% (v/v)) for 1 hour to obtain the silanol. Then, 10 mg of copper nanoparticles were dispersed in 10 mL of silanol to functionalise the NPs with the coupling agent. This dispersion was subsequently coated onto the fabric substrate. In the second approach, the substrates were coated with 1% (w/w) PDMS in THF (at an elastomer and curing agent ratio of 1:10). After coating, the PDMS-containing substrates were subjected to heat treatment at 100 °C for 35 minutes. This may be done using dip coating or spray coating.

- Dip coating may involve the following steps (fabric substrate as a non-limiting example).
1. The surface of the fabric substrate is modified by deposition of a layer of organosilicon materials that may or may not be a hybrid layer of organosilicon materials. The surface may optionally be modified by plasma activation prior to the deposition of the organosilicon materials. Whether or not the plasma treatment is necessary depends on the specific substrate used. For example, plasma treatment is necessary for alkene-based polymers or fluoropolymers.
 2. The copper nanoparticles may be functionalized with various organosilicon compounds. This functionalization could be needed to improve the compatibility of the nanoparticle with the surface. In the examples below, no functionalisation of the organosilicon compounds was conducted unless otherwise explicitly specified.
 3. The copper nanoparticles (any particle size) may be dispersed in organic solvents such as methanol, ethanol or isopropyl alcohol at a ratio of 1 mg nanoparticle per mL of solvent per cm² of fabric. As will be appreciated, different nanoparticle amounts can be used if desired.
 4. The copper nanoparticles may be dispersed in an appropriate solvent using an ultrasonic bath for up to 20 minutes at room temperature, to produce a homogenous dispersion.

5. The modified fabric substrate may be transferred into the functionalized nanoparticle dispersion container. The substrate and dispersion may be mixed for 60 minutes, for example with the aid of a magnetic stirrer at 300-800 rpm.
6. The nanoparticle-coated fabric may be cleaned with an appropriate solvent to remove excess or unbound nanoparticles. The solvent used to remove excess or unbound NPs should be the same as the solvent used to disperse the NPs. Examples of solvents that can be used include methanol, ethanol, and isopropyl alcohol.
7. The cleaned nanoparticle-coated fabric may be dried in an oven at 100°C for at least 60 minutes. This process allows the evaporation of solvents and promotes the formation of chemical bonds between the nanoparticle formulation and the fabric.
8. Optionally, the resulting material may be coated with the plastic adhesive by spraying of the pre-prepared solution mentioned above, which is then allowed to dry before use.
- 15 Spray coating may use steps 1-4 as described above for dip coating, followed by the below.
- 9(a). The homogeneous nanoparticle dispersion may be transferred into the spray gun's mixing cup and coupled to the spray gun body. Atomizing heads with different nozzle diameter sizes can be used.
- 9(b). The coating may be sprayed on the modified fabric substrate at a constant distance followed by drying with heat gun. This process allows the evaporation of solvents and promotes the formation of chemical bonds between the nanoparticle formulation and the fabric. Several layers of coating can be applied using spray coating.
- 9(c). Optionally, the resulting material may be coated with the plastic adhesive by spraying of the pre-prepared solution mentioned above, which is then allowed to dry before use.

It will be appreciated that steps 9(a) to 9(c) immediately follow in sequence from step 4 of the method described above as an alternative to steps 5-8.

- 30 While the above processes refer to a fabric substrate, similar methods may be used with other substrates, or these methods may be adapted accordingly.

Nanoparticle and fabric characterization: The yCu and aCu nanoparticles, in both pristine particle form and particle-functionalized fabrics, were characterized with a field-emission scanning electron microscope (JEOL 7800F Prime) with an energy-dispersive X-ray spectroscopy (EDS) detector (Ultim Max, Oxford Instruments). The accelerating voltages used for SE imaging and EDS analysis were 5 kV and 20 kV, respectively. To minimize the effects

of charging, the non-conductive fabrics were first coated with an ultrathin layer of Pt. The X-ray photoelectron spectroscopy (XPS) spectra were collected using a Kratos AXIS Supra (Al K α source, 225 W) over an analysis area of 700 μm x 300 μm with a take-off angle of 90°. The energy-loss spectra were recorded on a JEOL 2100F transmission electron microscope with an accelerating voltage of 200kV and a Gatan imaging filter. The Auger Electron Spectroscopy measurements were conducted on a JEOL JAMP-7830F machine equipped with a field-emission electron gun and a hemispherical analyzer. The AES analysis was performed at an acceleration voltage of 10 keV and a probe current of 10×10^{-9} A. The sample was tilted at 30° throughout the analysis and the analysis area was approximately 15 x 15 μm^2 .

Spray-coating of the fabric materials: aCu (1% w/w) was dispersed in IPA and sonicated in an ultrasonic bath for two minutes. The same method was employed for the yCu powder. The plastic adhesive (2.5% w/w) was dissolved in a mixture of acetone and IPA at a 1:4.5 weight ratio. PDMS (1% (w/w)) was solubilised in THF (at an elastomer and curing agent ratio of 1:10). Fabrics cut into squares with an area of 930 cm^2 were spray-coated with the copper dispersions, the adhesive solution, and PDMS solution using a 3M Accuspray™ spray gun. The following steps were adopted to coat the substrates (using copper NP as example):

1. Load the copper NP (yCu or aCu) dispersion into the spray gun.
2. Activate trigger and spray 3 inches away from the substrate in a horizontal direction right to left.
3. Release trigger at the left edge of the substrate.
4. Move centre nozzle 1.25 inches down.
5. Reactivate trigger and spray in a left to right motion.
6. Release trigger at the right edge of the substrate.
7. Continue pattern until the whole substrate has been coated.

The steps above describe one pass of coating. For the second pass, spraying of the NPs dispersion was continued in the vertical direction and the same pattern as the horizontal spray was followed, but top to bottom and bottom to top with moving 1.25 inches to the right for every pass. Lastly, the NP-coated substrate was dried with a heat gun on heat mode approximately 3-5 inches away with continuous sweeping motion until the substrate was dry. The same spray procedure for adhesive was followed. In total, two passes of NPs dispersion and two passes of adhesive were applied. The optional layer of adhesive was sprayed last to prevent the loss of nanoparticles, and more importantly to impede the inhalation of airborne particulate matter. The same spray-coating protocol for PDMS coating was applied. However, the order of application was first the PDMS solution, and secondly, the coating with the NPs dispersion.

Dip-coating of the fabric materials: 10 mg of aCu were dispersed in 10 mL of ethanol and sonicated in an ultrasonic bath for 15 minutes. Alternatively, 10 mg of aCu were dispersed in 10 mL of silanol and sonicated in an ultrasonic bath for 15 minutes. The same methods were employed for the yCu powder. PDMS (1% (w/w)) was solubilised in THF (at an elastomer and curing agent ratio of 1:10). Fabrics cut into squares with an area of 9 cm² were dip-coated in the PDMS solution for two minutes and oven-dried at 100 °C for 35 minutes. Then, the PDMS-coated substrate was dip-coated in the NPs dispersion and stirred (aided by a magnetic bid at 300 rpm) for one hour in a screw-cap glass vial. Lastly, the PDMS-NPs-coated substrate was removed from the vial, washed with ethanol and oven-dried at 100 °C for one hour. When coating the substrates with NPs functionalised with coupling agents, the PDMS coating was not applied.

Bacterial strains: The bacterial strains used in this study include carbapenem resistant hypervirulent *Klebsiella pneumoniae* ENT646, laboratory stock strain *Pseudomonas aeruginosa* PAO1, *Escherichia coli* AS1.90, *Staphylococcus aureus* AS1.89 and *Staphylococcus aureus* ATCC strain SA29213. Bacterial strains were routinely cultured and maintained on lysogeny broth (LB) agar.

Preparation and treatment of fabrics with bacteria: Circular discs of 6 mm diameter were prepared with a 6 mm hole punch and sterilized via ultraviolet (UV) exposure for at least an hour. Fabric samples were pre-wet with 5 µL of sterile deionized H₂O before inoculation with 5 µL of bacterial cultures at various doses for a defined contact time.

Determination of antibacterial activity: After inoculation of bacterial cultures onto the fabrics for the indicated duration, fabrics were clipped to the lid of an Eppendorf tube and centrifuged at maximum speed for 1 minute, retrieving the liquid absorbed by the fabrics. The fabrics were then washed in sterile phosphate-buffered saline (PBS; Vivantis) to retrieve adherent bacteria, if any. The resultant solution from the fabrics and wash solutions were serially diluted and plated on LB agar and incubated at 37 °C overnight. Percentage killing was calculated by comparing bacterial numbers obtained from fabrics with yCu or aCu to the average bacterial counts obtained from control fabrics F#1, F#2 or F#3, according to the formula: 100 - (counts from the fabric of interest/average counts from control * 100).

For experiments with repeated inoculation of bacteria, 10⁴ CFU of *Klebsiella pneumoniae* ENT646 were inoculated sequentially, as described above, thrice a day for five consecutive days at 0, 3, 7, 24, 27, 31, 48, 51, 55, 72, 75, 79, 96, 99, 103 hours and on day 7, at 144 hours.

At each timepoint, bacteria were retrieved from fabrics for the determination of bacterial numbers and percentage killing as described above.

Fabrics were inoculated with 10^8 CFU of *Klebsiella pneumoniae* ENT646 for 45 seconds or 1 hour to image the interaction of the bacteria with the NP-coated fabrics. Fabrics were then fixed in 4% paraformaldehyde for 15 minutes and washed in 10 mM glycine twice before three washes in deionized H₂O. Fabrics were then dried overnight in a desiccator cabinet and imaged with the SEM.

10 *Examining DNA fragmentation:* Bacterial genomic DNA extraction was performed according to Weaver *et al.*'s methodology (L. Weaver, J. O. Noyce, H. T. Michels, C. W. Keevil, *J Appl Microbiol* 2010, 109, 2200), with minor modifications. Briefly, five fabric samples were inoculated with 10^8 CFU of *K. pneumoniae* ENT646. After 45 seconds or 1 hour at room temperature, fabrics were transferred to sterile PBS with 20 mmol⁻¹ EDTA and vortexed for 30
15 seconds. Bacterial cells were pelleted by centrifugation at 4000 g for 5 minutes. Bacterial genomic DNA was isolated and purified with GenElute™ Bacterial Genomic DNA kit (Sigma). Isolated DNA was examined via gel electrophoresis.

Oxidative DNA damage analysis: To examine the amount of oxidative damage in DNA, the
20 fabric was pre-wet with 10 mM Tris-HCl buffer pH 8.0 before the addition of 50 µg of deoxyribonucleic acid (DNA; Sigma). After 45 seconds or 1 hour, fabrics were clipped to the lid of an Eppendorf tube and centrifuged at maximum speed for 1 minute. DNA retrieved was hydrolyzed and examined via LC-MS for levels of 8-hydroxy-2'-deoxyguanosine (8OHdG).

25 *Statistical Methods:* Statistical significance of 95% confidence between three or more groups were determined by ANOVA followed by Tukey's test. All statistics were conducted using the GraphPad Prism software. Statistical significance is indicated as follows: *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001

30 Example 1: Preparation of copper nanoparticle-coated fabrics

The systematic generation and characterization of the copper NP-coated fabrics is depicted schematically in Figure 1a. In the first instance, the adhesive (3M 2262) and copper nanoparticles are dispersed in their respective solvents (as is the adhesive, when used) in
35 cartoon section (1). The various materials are then spray-coated onto the substrate in cartoon section (2) – the order of spraying is the copper nanoparticles, followed by (when desired) the adhesive material. The resulting materials can then undergo an antibacterial assay,

whereupon they are exposed to various bacterial strains (cartoon section (3)), which may result in the bacterial undergoing oxidative stress and genomic DNA fragmentation.

Two species of copper were used, a commercial cuprous oxide (Cu_2O), henceforth named yCu, and commercial metallic copper NPs, henceforth named aCu. Both formulations were spray-coated on three different fabric materials (Fabric #1, Fabric #2 and Fabric #3, as discussed above) commonly used to fabricate facemasks and filters in air purification units present in many different systems such as heating, ventilation, and air conditioning systems in hospitals and airplanes. Spray-coating of the three fabric materials with copper NPs dispersion (yCu or aCu) and adhesive solution was performed by following the spray-coating protocol described above except no PDMS coating was applied.

Example 2: Characterisation of copper nanoparticle-coated fabrics

The formulations of copper NPs were characterized before and after spray coating on the fabrics (prepared in Example 1). Their antibacterial activities, morphological effects on the bacteria, the amount of oxidative damage produced and bacterial genomic DNA fragmentation after bacterial interaction were evaluated.

Individual, well dispersed NPs exhibit very high surface-area-to-volume ratios, which is highly advantageous in applications that require direct contact between the NPs and the pathogens. One of the mechanisms proposed involves the oxidation state of the copper species, and this has shown to have a significant impact on antibacterial activity. The state of the copper NPs were assessed before and after coating onto the various fabrics to understand the important underlying factors contributing to effective antibacterial behaviour. The size, shape and oxidation state of the copper NPs were first determined (Figure 1 (b) – (k)), and the analysis results are tabulated (Table 1).

		XPS			
		Cu	Cu₂O	CuO	Cu(OH)₂
yCu ^a	Cu 2p _{3/2} binding energy [eV]	-	932.3	-	934.3
	Atomic concentration [%]	-	80.63	-	19.37
yCu ^b	Cu 2p _{3/2} binding energy [eV]	-	932.2	-	934.3
	Atomic concentration [%]	-	8.15	-	91.85
aCu	Cu 2p _{3/2} binding energy [eV]	932.5	-	-	-
	Atomic concentration [%]	100.00	-	-	-
		AES			
		Cu	Cu₂O	CuO	
yCu	Cu LMM kinetic energy [eV]	-	913.1	914.3	
	Atomic concentration [%]	1.0 ± 1.2	95.3 ± 5.1	3.8 ± 4.0	
aCu	Cu LMM kinetic energy [eV]	915.1	-	-	
	Atomic concentration [%]	88.5 ± 3.1	0.7 ± 1.6	10.8 ± 1.7	
		EELS			
		Cu	Cu₂O	CuO	
yCu	Cu L ₃ edge energy [eV]	-	937.9	938.0	
aCu	Cu L ₃ edge energy [eV]	936.7	-	-	

^a) Fresh sample; ^b) Sample left in ambient for at least a month.

Table 1. Cu 2p binding energies, Cu LMM kinetic energies, and Cu L₃ edge energies from the XPS, AES and TEM-EELS spectra, respectively, for the yCu and aCu nanoparticles.

- 5 The scanning electron microscopy (SEM) image of the yCu (Cu₂O) NPs reveals hierarchical structures (Figure 1b). Small NPs with an average size of about 150 nm were observed to form large aggregates. On the other hand, aCu NPs show a smaller average size of about 50 nm with a regular spherical shape, and due to their high surface energy form large aggregates or clumps (Figure 1g). The inherent small size of the nanoparticles gives rise to a higher surface-to-volume ratio and the resultant rough nano-level surface topology, and its high surface energy fosters direct contact and interaction with the infectious agents. However, the tested nanoparticles were heavily agglomerated micron size aggregates (~1-15 μm) and no individual nanoparticles were present. Using a combination of characterization techniques such as X-ray photoelectron spectroscopy (XPS), X-ray diffraction (XRD), Auger electron spectroscopy (AES), and transmission electron microscopy electron energy-loss spectroscopy (TEM-EELS), the oxidation state of both copper species can be determined. The Cu 2p core-level spectra of both yCu and aCu show very similar features, i.e., doublets with spin-orbit splitting of ~ 19.8 eV, with the yCu showing additional weak satellite features (Figure 1d and

i). Peak analysis was conducted for the Cu 2p_{3/2} component using Gaussian–Lorentzian line shapes as this will enable the differentiation of Cu species in both copper samples. For yCu, the deconvolution of the core-level Cu 2p_{3/2} signal yields a main peak at 932.3 eV and another small peak at 934.3 eV, corresponding to Cu₂O and Cu(OH)₂ phases, respectively (Figure 1d).

5 The satellite features observed are attributed to the hydroxide phase. On the other hand, for aCu, the Cu 2p_{3/2} peak is at 932.5 eV (Figure 1i). The extracted XPS Cu 2p_{3/2} core-level binding energies are in the increasing order Cu(I) < Cu(0) < Cu(II), which is in good agreement with the literature. From a quantitative analysis, about 80.6% of the whole sample consists of Cu₂O. Without wishing to be bound by theory, the residual Cu(OH)₂ is believed to be a

10 metastable phase, which formed upon the interaction between Cu ions and the hydroxyl groups on the surface of Cu₂O particles. In fact, XPS analysis of an aged yCu sample (Figure 5) reveals that Cu(OH)₂ could account for as much as 91.9% of the total Cu, presumably due to significant water absorption on the oxide surface at high humidity. From this observation, it can also be inferred that yCu was likely to have high phase purity (~100%) in the absence of

15 surface hydroxyl groups. Chemical state identification provided by Auger electron spectroscopy (Figure 7) also agrees reasonably well with XPS and the XRD patterns (Figure 6). The fitting of the differentiated AES spectra shows peaks at 915.1 eV in aCu corresponding to metallic Cu and at 913.1 eV and 914.3 eV in yCu, corresponding to the Cu₂O and CuO phases, respectively. The AES spectra reveal a main phase of Cu₂O in yCu with an atomic

20 concentration of 95.3% (Figure 1e), while aCu primarily consists of metallic Cu species. The discrepancy in the composition of yCu provided by XPS and AES can be attributed to the difference in the sampling volume and depth of analysis. As Cu 2p photoelectrons and Cu LMM Auger electrons have different kinetic energies, i.e., 530–550 eV and 900–920 eV, respectively, XPS is expected to provide more surface-sensitive information from the sample.

25 It is also worth mentioning that the intensity of the O KLL signal is stronger in yCu than in aCu, as shown in the AES survey scan spectra (Figure 7). Figures 1f and k show the two copper *L*_{2,3} edges obtained from yCu and aCu, respectively, using EELS. The two principal features, the *L*₃ and *L*₂ edges, arise from the spin-orbital splitting of the 2*p* core hole and are separated by about 20 eV. The full black line in Figure 1f shows an asymmetric peak from one region of

30 yCu and a feature marked by an arrow in the vicinity of the *L*₂ edge. The *L*₃ edge in this spectrum is located at 937.9 eV. Another EELS edge (blue dotted line, Figure 1f) collected from a nearby region of yCu shows a different profile, with the *L*₃ edge located at 938.0 eV. For aCu, shown in Figure 1k, the *L*₃ edge is located at 936.7 eV and the EELS edge shows no white lines. The decrease in white line intensity is due to a filled 3*d* shell. The spectral

35 shape and the relative position of the *L*₃ edges in yCu and aCu allow us to further confirm the presence of Cu₂O and CuO in yCu, and metallic copper as the main constituent of aCu.

The combination of XPS, AES, and TEM-EELS enables a conclusion that yCu is mainly composed of Cu_2O (Cu^+), and in aCu, the copper exists almost exclusively in the metallic form (Cu^0).

- 5 To investigate the nanoparticle distribution, SEM images and EDS maps of the copper-coated fabrics were collected at 100 $\times$ and 500 $\times$ magnification. The atomic concentration (at%) determined using EDS mapping does not vary significantly at different magnifications (Table 2).

		Cu (%)	
		100x	
		F#1	F#2
yCu		4.2 $\pm$ 0.2	10.4 $\pm$ 0.9
aCu		8.1 $\pm$ 0.5	9.5 $\pm$ 0.6
		500x	
		F#1	F#2
yCu		4.7 $\pm$ 0.6	9.3 $\pm$ 1.3
aCu		6.9 $\pm$ 0.4	9.4 $\pm$ 0.8

Table 2. Values of the atomic concentration (%) of copper obtained from the SEM-EDS maps at low (100x) and high (500x) magnification.

- 15 Fabric #1 (F#1) is a 55/45% cellulose/polyester fabric blend with wide and flat cellulose stripes, and fabric #2 (F#2) is a 70/30% rayon/polyester fabric blend with polymer fibres sparsely distributed which may improve the breathability when used as the filter layer in face masks. Both yCu and aCu can be easily identified on the SEM images and are uniformly distributed in the form of large 1-15-micron size aggregates on the filter materials, Figure 2. The SEM
- 20 images and EDS maps collected at 100 $\times$ magnification can be found in Figure 4. The aCu appears homogeneously distributed on both fabrics, with a coverage that is approximately twice that of the Cu on fabric F#1. This can be attributed to the structure of the pristine fabrics and the size of the yCu particles. Though yCu particles are rather large, the features present on the pristine fabric F#2 (Figure 2e) facilitated the loading of yCu. On the one hand, the
- 25 polymer fibers in F#2 are sparsely distributed, which contribute to enhanced airflow. On the other hand, an interdigital membrane-like structure facilitated the anchoring of NPs by providing an additional surface for particle bonding, hence contributing to a more robust coating.
- 30 To investigate the biocidal effect of the yCu and aCu NPs, the antibacterial activity of the copper-coated fabrics against Gram-negative bacteria, carbapenem-resistant cum hypervirulent *Klebsiella pneumoniae* ENT646 and *Pseudomonas aeruginosa* lab strain PAO1,

and Gram-positive bacterium, methicillin-sensitive *Staphylococcus aureus* 29213, was investigated. Various copper-coated and their corresponding control fabrics were inoculated with 10^4 CFU bacteria for 45 seconds before the enumeration of bacterial counts. The killing efficiencies were determined by comparison of bacterial numbers obtained from the copper-coated fabrics to the average bacterial counts obtained from control fabrics F#1, F#2, or F#3. For *S. aureus*, a solid commercial vinyl film F#3 (AIVA) was used to determine biocidal effects of the NPs as the low bacterial counts retrieved from the control fabrics F#1 and F#2 (Figure 8d) would skew killing efficiency calculations. Against all three types of bacteria, both yCu and aCu copper fabrics had significantly reduced bacterial numbers retrieved as compared to control fabrics (Figure 3e-g and Table 3), with yCu- and aCu-coated fabrics exhibiting average killing efficiencies of >93% and >84%, respectively.

<i>Klebsiella pneumoniae</i> ENT646		
	F#1	F#2
yCu	99.91 $\pm$ 0.06	100.00 $\pm$ 0.00
aCu	84.64 $\pm$ 1.28	100.00 $\pm$ 0.00
<i>Pseudomonas aeruginosa</i> PAO1		
	F#1	F#2
yCu	93.58 $\pm$ 3.80	97.44 $\pm$ 0.89
aCu	86.71 $\pm$ 9.71	99.99 $\pm$ 0.01
<i>Staphylococcus aureus</i> 29213		
	F#3	
yCu	95.49 $\pm$ 1.92	
aCu	95.55 $\pm$ 3.77	

Table 3. Killing efficiencies of various fabrics on *Klebsiella pneumoniae* ENT646, *Pseudomonas aeruginosa* PAO1 and *Staphylococcus aureus* SA29213.

No obvious difference in killing efficiencies was observed between yCu- and aCu-coated fabrics at a higher dosage of 10^5 CFU *K. pneumoniae* (Figure 8a). However, at 10^6 CFU *K. pneumoniae*, yCu exhibited poorer killing efficiency as compared to aCu when coated on fabric F#1 whereas no difference was observed when coated on Fabric F#2 (Figure 8b). The more efficient killing may be associated with the higher surface coverage of aCu on fabric F#1 detected from SEM-EDS, compared to yCu-coated fabric F#1 as observed in the SEM-EDS compared to yCu (Figure 2 and Table 2). These suggest that in addition to the oxidation state of the copper nanoparticle, the nanoparticle concentration and the base fabric can affect the

antibacterial efficacies of the copper-coated fabrics. Further study with the NPs was performed using *K. pneumoniae* ENT646 and fabric F#1 as the base substrate. When coated on fabric F#1, yCu displayed sustained ability to kill over cumulative inoculations with 10^4 CFU *K. pneumoniae* across the duration of 144 hours while aCu-coated fabrics had a gradual fluctuating decline in killing (Figure 3h-i). This demonstrates that yCu is more robust than aCu. To understand this difference in bacterial killing, we studied the effect of the addition of deionized water onto the copper-coated fabrics using XPS (Figure 9) since the bacteria were dispersed in deionized water for inoculation. The water was added onto the copper-coated fabrics for 144 hours, and three data points for analysis were selected. The addition of water onto yCu-coated fabric showed a negligible effect on the atomic concentration of $\text{Cu}(\text{OH})_2$ present on the surface of the fabric. In contrast, the aCu-coated fabric revealed a decrease of $\text{Cu}(\text{OH})_2$ with the exposure time (Table 4).

XPS					
			Cu	Cu_2O	$\text{Cu}(\text{OH})_2$
	No- H_2O		-	41.25	58.75
yCu	Initial- H_2O	Atomic concentration [%]	-	43.36	56.64
	Final- H_2O		-	42.28	57.72
	No- H_2O		31.83	-	68.17
aCu	Initial- H_2O	Atomic concentration [%]	30.68	-	69.32
	Final- H_2O		53.59	-	46.41

Table 4. Atomic concentrations of Cu, Cu_2O and $\text{Cu}(\text{OH})_2$ determined using XPS Cu 2p binding energies for the yCu- and aCu-coated fabrics before and after addition of deionized water. The results correspond to three time points: dried fabric with no water (No- H_2O), after the first addition of water (Initial- H_2O), and after the last addition of water at $t=144$ hours (Final- H_2O).

To determine the factors contributing to the mode of action of yCu and aCu and the possible difference in robustness, we examined bacterial morphology via SEM. A high dosage of 10^8 CFU *K. pneumoniae* and a prolonged duration of 1 hour was selected to increase the chances of visualizing the effects of the fabrics on bacteria. At this inoculating dose, both yCu- and aCu-coated fabrics exhibited approximately 47% to 62% killing (Figure 8c). Indentations were observed on the bacterial surface after interaction of 10^8 CFU *K. pneumoniae* with both yCu- and aCu-coated fabrics F#1 for 1 hour (Figure 3c-d), suggesting bacterial cell surface structural damage. Additionally, bacterial DNA harvested after interaction of *K. pneumoniae* with yCu- and aCu-coated fabrics for 45 seconds and 1 hour resulted in a smeared band during gel electrophoresis, indicative of rapid DNA fragmentation within 45 seconds (Figure

3j). As oxidative stress can result in DNA fragmentation, we assessed the levels of 8-oxo-2'-deoxyguanosine (8OHdG), a marker of oxidative damage to deoxyguanosine (dG), after DNA contacts the copper-coated fabrics. Significantly higher levels of 8OHdG per 10,000 dG were detected after DNA interaction with both yCu- and aCu-coated fabrics compared to control fabric F#1 at 45 seconds and 1 hour, signifying that both copper NPs induce oxidative stress rapidly, Figure 3k. Between the two copper coated fabrics, aCu-coated fabrics resulted in greater oxidative damage of dG. This correlates with the lower atomic concentration of yCu (4.2%), which is approximately about half of that of aCu (8.1%) on F#1 (Table 2). These results indicate that both copper materials are capable of inducing bacterial structural damage and oxidative stress. However, additional antibacterial mechanisms may be involved as yCu displays equally high killing efficiencies and greater robustness despite lower nanoparticle surface coverage on fabric F#1 and the generation of oxidative damage.

These results demonstrate the high antibacterial efficacies of yCu and aCu copper-coated fabrics against Gram-positive bacteria *S. aureus* and Gram-negative bacteria *K. pneumoniae* and *P. aeruginosa*. This reflects a broad spectrum of antibacterial activity where the copper-coated fabrics are likely effective against a wide variety of bacteria.

Based on DNA degradation and modification of deoxyguanosine as markers for oxidative damage, it is reasonable to propose that bacteria underwent rapid and significant oxidative damage within 45 seconds after contact with both yCu and aCu. This strongly indicates that ROS are generated by the copper-coated surfaces. Given that ROS also damage membrane lipids and proteins, the deformation and indentations observed on the bacterial surface after contact with yCu and aCu could potentially be a result of ROS production. The membrane deformation could also result from attractive electrostatic forces between the bacteria and the particles that potentiate the bacterium-nanoparticle interaction, hence resulting in increased membrane tension force. Additionally, non-translating NPs have been previously reported to induce mechanical damage via an increase in membrane tension resulting in membrane deformation, with larger clusters demonstrating more membrane stretching and disruption. As discussed earlier, the presence of $\text{Cu}(\text{OH})_2$ on the surface of the copper-coated fabrics is likely from a metastable phase, presumably due to significant water absorption on the oxide surface at high humidity. However, the aCu-coated fabric revealed a relatively high atomic concentration (68.2%) of Cu^{2+} before water treatment (Table 4). These results suggest that the surface of the aCu-coated fabric had oxidized to Cu^{2+} easily after being coated which is somewhat expected because copper oxidizes to its most stable phase, CuO. The cumulative addition of water showed a decrease in the hydroxide signal, probably related to the dissolution of the native oxide, which compromised the killing efficiency. Notably, the yCu-

coated fabric showed no effect on water addition, hence resulting in a stable and long-lasting killing efficacy. Moreover, the “contact-killing” effect of copper may depend on the uniformity of the coating layer and the number of NPs on the surface of the fabric materials that makes contact with the bacteria. In line with this, larger-sized yCu NP-coated fabrics were more robust in maintaining high levels of antimicrobial activity during repeated exposure to bacteria, despite lower surface coverage of yCu and lower induction of oxidative stress. It is probable that the larger particle size of yCu mechanically damages membranes with concomitant ROS production.

Overall, the results demonstrate that both copper-coated fabrics possess rapid high antimicrobial activity against a broad spectrum of bacteria. Multiple mechanisms including ROS generation and mechanical damage likely contribute to the efficiency and robustness of the copper-coated fabrics.

While both species of copper are capable of inducing bacterial structural damage and oxidative stress, Cu⁺ (yCu) displays greater robustness despite lower nanoparticle surface coverage and generation of oxidative stress. Since both copper species have been prepared using the same deposition route and compared on the same platform, it can be concluded that Cu⁺ is most effective for robust biocidal coatings.

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Example 3: Washing and durability of the composite material

For the durability on extensive washing, cotton fabrics dip-coated with PDMS and yCu NPs (prepared by following the dip-coating protocol above) were added into an aqueous solution of Triton-X 100 (1 g/L) in ultrapure water (18 mΩ) at a liquor ratio of 50:1 ml/g. The fabrics were washed in this aqueous solution for 6 hours in an ultrasound bath (275 W) at 45 °C.

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The 6-hour washing duration was performed as approximately 120 washing cycles (3 min/cycle).

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Results are shown in Figure 10. (a) and (b) show (1) SEM images, (2) EDX maps, (3) contact angle images for the composite material before (a) and after (b) washing in an aqueous solution of Triton X-100 at 45 °C for six hours. (c) shows FTIR-ATR spectra of the composite material.

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The results show that the composite material is substantially unchanged after washing.

Example 4: Comparison of dip- and spray-coating

The meltblown polypropylene filter fabric material was cut into squares with an area of 9 cm² for the coating procedures. The meltblown polypropylene filter fabric material was dip-coated with yCu (Cu₂O) dispersed in 10 mL of ethanol by following the dip-coating protocol described above except the fabric material was coated with NPs only. The meltblown polypropylene filter fabric material was spray-coated with yCu (Cu₂O) dispersed in 10 mL of ethanol by following the spray-coating protocol described above except the fabric material was coated with NPs only, the yCu dispersion was sonicated in an ultrasonic bath for 15 minutes, and the NP-coated substrate was oven-dried at 100 °C for one hour. In total, two passes of copper NPs dispersion were applied.

Figure 11 shows a comparison of a meltblown polypropylene filter (substrate) coated with a copper (I) oxide formulation. Column (a) shows the filter without formulation. Column (b) shows the dip-coated filter. Column (c) shows the spray-coated filter. Rows 1, 2 and 3 show optical photographs of the filters, scanning electron micrographs, and energy-dispersive X-ray maps, respectively. (a3) shows the carbon element, and (b3) and (c3) show the copper element. The scalebar is 50 µm.

From these images the amount of copper present on the surface of the composite material was calculated. The dip coated composite material was calculated to comprise 1.5 ± 0.5 wt. % of Cu₂O, while the spray coated composite material was calculated to comprise 9.5 ± 0.2 wt. % Cu₂O.

This shows that it is easier to provide composite materials having substantially higher amounts of nanoparticles comprising Cu₂O by spray coating than by dip coating.

Figure 12 shows an SEM image of fabric fibres coated with nanoparticles (dip coating).

Example 5: Assessment of antibacterial activity of different copper nanoparticle formulations

To evaluate the antibacterial properties of different copper oxide species, the nanoparticle size effect and the presence of linkers, five copper NP formulations (1 mg/mL) in ethanol were coated onto a cotton substrate, and pristine cotton was used as a reference. The five samples were labelled from A to F, and their composition is as follows: A) pristine cotton, B) CuO-MPTMS-coated cotton, C) Cu₂O-HDTMS-coated cotton, D) CuO-coated cotton, E) Cu₂O-

coated cotton (NP size around 100 nm), and F) Cu₂O-coated cotton (NP size around 7 microns).

The copper nanoparticles were first functionalized with the small silane molecules (1% hydrolysed silane) and then coated on cotton for samples B and C by following the dip-coating protocol described above except no PDMS coating was applied. The CuO nanoparticles in sample B was functionalised with 1% hydrolysed MPTMS while the Cu₂O nanoparticles in sample C was functionalised with 1% hydrolysed HDTMS. Samples D, E, and F were coated on cotton with the respective copper NP dispersed in ethanol, by following the dip-coating protocol described above except no PDMS coating was applied. After coating, all the samples were heat-treated for 60 minutes at 100 °C. The samples were cut into circular fabrics of 6 mm diameter and inoculated with 10⁴ CFU of carbapenem-resistant hypervirulent *Klebsiella pneumoniae* ENT646 for a duration of 30 minutes before the enumeration of bacterial counts.

Out of five samples, only the fabric coated with Cu₂O nanoparticles (sample E) revealed a significant reduction in antibacterial counts after 30 minutes of interaction with the bacteria (Figure 13a-b). Sample D showed negligible effect in killing the bacteria, which supports previous studies where Cu₂O in sample E was found to be more reactive than CuO, hence resulting in more bacterial damage. Though sample F is also composed of Cu₂O nanoparticles, the comparatively larger size of its particles reduces the surface-area-per-volume ratio, which is believed to be the reason for its poor antibacterial performance. Lastly, the copper NPs were functionalised with small silane molecules to enhance the fabric-NP compatibility. Silanes with different polarities were used to tune the surface energy of the substrate, but in both cases, their presence was not beneficial to the bacterial killing. These two small molecules might mask the antibacterial properties of copper nanoparticles or even reduce the interactions between the bacterium and the NP. One should not rule out the usage of small silane molecules, but further investigations are recommended to functionalise the copper nanoparticles in such a way that their inherent antibacterial properties are preserved or even enhanced.

Based on the results above, sample E was then assessed for antibacterial properties against *Escherichia coli* AS1.90 and *Staphylococcus aureus* AS1.89 using the standard test GB/T 21866-2008. The Cu₂O-coated cotton showed an antibacterial rate >99.99% (log₄ reduction) against both Gram-negative and Gram-positive bacteria strains tested (Figure 13c).

Example 6: Copper nanoparticle formulations for dip-coating of various fabrics

Fabrics dip-coated with PDMS and copper NPs were fabricated by following the dip-coating described above. 10 mg of yCu (NP1) or Cu₂O powder with particle size ~7 micrometres (NP2)
5 was dispersed in 10 mL of ethanol and sonicated in an ultrasonic bath for 15 minutes. Then, the copper nanoparticle dispersion (NP1 or NP2) was dip-coated onto different woven and non-woven PDMS-coated fabrics (cotton, PET, melt-blown PP and spunbond PP).

The results showed that the coating is homogeneous irrespective of the substrate material,
10 and the fibers are densely functionalized with the copper nanoparticles (Figure 14).

CLAIMS

1. A composite material for killing a microbe on contact comprising:
a substrate material having a surface;
a silicon-containing material attached to the surface of the substrate material; and
a plurality of nanoparticles comprising Cu₂O attached to the silicon-containing material.
2. The composite material according to Claim 1, wherein the substrate material is selected from one or more of a glass, a polymer, and a fibrous material.
3. The composite material according to Claim 2, wherein:
(aa) the fibrous material is selected from one or more of the group consisting of cotton, linen, silk, polyester (such as woven- or non-woven polyester), polypropylene (such as a meltblown polypropylene or spun bond polypropylene), polyurethane, cellulose, cellulose/polyester blend, rayon, optionally wherein the fibrous material is in the form of a woven or non-woven fabric; and/or
(bb) the fibrous material comprises a plurality of fibres and the silicon-containing material coats at least part of some of the plurality of fibres.
4. The composite material according to any one of the preceding claims, wherein the silicon-containing material comprises a silicon-containing polymer or a silicon-containing small molecule, optionally wherein the silicon-containing polymer comprises a siloxane polymer.
5. The composite material according to Claim 4, wherein:
(AA) the silicon-containing small molecule is selected from one or more of tetraethylorthosilicate (TEOS), aminoethylaminopropyltrimethoxysilane (AEAPTMS), 3-aminopropyltriethoxysilane (APTES), methacryloyloxypropyltrimethoxysilane (MPTMS), hexadecyltrimethoxysilane (HDTMS), n-octadecyltriethoxysiloxane (ODTMS); or
(BB) the silicon-containing polymer is polydimethylsiloxane (PDMS).
6. The composite material according to any one of the preceding claims, wherein the silicon-containing material has a water contact angle of from 130° to 150°, optionally wherein the silicon-containing material has a water contact angle of from 134° to 145°, such as from 134.5° to 140°.
7. The composite material according to any one of the preceding claims, wherein the composite material is porous.

8. The composite material according to any one of the preceding claims, wherein the composite material is washable.

9. The composite material according to Claim 8, wherein after being subjected to a 6-hour wash cycle in an aqueous solution of Triton-X 100 (2-[4-(2,4,4-trimethylpentan-2-yl)phenoxy]ethanol) (1 g/L) in ultrapure water (18 mΩ) at a liquor ratio of 50:1 ml/g in an ultrasound bath (275 W) at 45°C, the composite material:

(ia) retains at least 90 mol %, such as at least 95 mol %, e.g. at least 99 mol % of the original amount of copper (I) oxide; and/or

(ib) retains a water contact angle of $\pm 10^\circ$, such as $\pm 5^\circ$, $\pm 2^\circ$, e.g. $\pm 1.5^\circ$, as compared to the water contact angle of the composite material before washing.

10. The composite material according to any one of the preceding claims, wherein less than 20% by weight of the initial loading of the nanoparticles comprising Cu_2O is lost from the composite material after being subjected to a 6-hour wash cycle in an aqueous solution of Triton-X 100 (2-[4-(2,4,4-trimethylpentan-2-yl)phenoxy]ethanol) (1 g/L) in ultrapure water (18 mΩ) at a liquor ratio of 50:1 ml/g in an ultrasound bath (275 W) at 45°C, optionally wherein less than 15%, less than 10%, less than 5%, less than 4%, less than 3%, less than 2%, or less than 1% by weight of the initial loading of the nanoparticles comprising Cu_2O is lost from the composite material after being subjected to a 6-hour wash cycle as described above.

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

(ai) the nanoparticles comprising Cu_2O have a mean diameter of from 10 to 500 nm, optionally from 20 to 300 nm, more optionally from 30 to 200 nm, for example from 40 to 150 nm, such as about 73.5 nm; and/or

(aii) the nanoparticles comprising Cu_2O form from 2 to 15 wt% of the total weight of the composite material, optionally wherein the nanoparticles comprising Cu_2O form from 3 to 13 wt%, such as from 4 to 11 wt%, such as about 4.9 wt% or about 9.5 wt%, of the total weight of the composite material.

12. A method of making a composite material according to any one of Claims 1 to 11, comprising the steps of:

(i) providing an intermediate material formed from:

a substrate material having a surface; and

a silicon-containing material attached to the surface of the substrate material;

and

- (ii) attaching a plurality of nanoparticles comprising Cu_2O onto the silicon-containing material.
13. The method according to Claim 12, wherein the intermediate material is formed by:
- (bi) providing a substrate material having a surface; and
 - (bii) attaching a silicon-containing material onto the surface of the substrate material, optionally wherein step (bii) comprises a preliminary step of activating the surface of the substrate material, such as by plasma surface activation.
14. The method according to Claim 12 or Claim 13, wherein step (ii) of Claim 12 is performed by dip-coating of the intermediate material into a dispersion of nanoparticles comprising Cu_2O .
15. The method according to Claim 12 or Claim 13, wherein step (ii) of Claim 12 is performed by spray-coating a dispersion of nanoparticles comprising Cu_2O onto the intermediate material,
- optionally wherein the atomic concentration of copper after spray-coating is from 5 to 15% as analysed by SEM-EDX maps at 500x magnification.
16. The method according to Claim 14 or Claim 15, wherein the dispersion of nanoparticles comprising Cu_2O is a dispersion of Cu_2O -containing nanoparticles in an organic solvent, optionally wherein the organic solvent is an alcohol (e.g. the organic solvent is selected from one or more of the group consisting of methanol, ethanol, and isopropyl alcohol).
17. The method according to Claim 16, wherein the ratio of nanoparticles comprising Cu_2O to organic solvent is from 0.5 to 5 mg nanoparticle/mL of solvent, such as from 0.7 to 2 mg nanoparticle/mL of solvent, such as about 1 mg nanoparticle/mL of solvent.
18. Use of a composite material as described in any one of Claims 1 to 11 as an antimicrobial surface.