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1. Introduction

1.1. About DEMAND Hub

The Data-Enabled Medical Technologies And Devices (DEMAND) Hub project is funded by the European Regional Development Fund as part of the European Structural and Investment Funds Programme 2014-2020 – Priority Axis1: Promoting Research and Development. The project is delivered by the University of Birmingham in strategic partnership with University Hospitals Birmingham NHS Foundation Trust. The DEMAND Hub programme will run until June 2023 and will support SME businesses in, or looking to enter, the healthcare market by delivering scientific services and commercial pathway support. In addition, there will be a strong focus on the utility of patient reported outcomes and healthcare data in guiding product design, testing and development, removing barriers for innovative businesses and stratifying product development.

The distinctive offering of the DEMAND Hub is characterised by the creation of new academic-clinical-innovation pathways between our existing Birmingham Health Partners expertise in medical technologies, health data and clinical trials, working with established medical technology companies as well as innovative digital SME companies, to enable them to access the opportunities within the regional medical and healthcare sector so as to facilitate development of new technologies across systems software, devices, algorithms, AI solutions and beyond.

1.2. Company and product description

Shaw and Partners have produced a hybrid cotton / copper material and wish to test the antibacterial activity it displays compared to cotton alone. It is hoped that this material will display antibacterial properties and may be used in bedding, dressings and wound care to reduce secondary infection.

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1.3. Report's objective

The objective of this report is to undertake a mini review of existing research published on PubMed regarding cotton / copper hybrids, their uses and potential antibacterial activity. In addition, we propose a protocol for testing the antibacterial activity for the material, and expected outcomes.

1.4. Reviewers' biography

This review was compiled by Adam McGuinness, who has 10+ years of experience assessing, analysing, and interpreting scientific data, utilising their interest in the development of the translation of ideas, technologies, and pharmaceutical products to bring them to market as effectively as possible.

2. Summary

This summary has analysed a number of scientific research papers regarding the manufacture and effectiveness of hybrid copper cotton materials with regards to antibacterial action. Due to the significant number of research papers available on this subject, an exhaustive review was not within the scope of the report, however, we have tried to produce a document that provides useful links for Shaw and Partners in their endeavors. In addition to the review, a simple testing methodology has been provided that would enable Shaw and Partners to perform preliminary testing of their product.

3. Report

3.1. Literature review

A literature search was performed using the scientific repository Pubmed (https://pubmed.ncbi.nlm.nih.gov/?term=copper+cotton+antibacterial) and the results assessed for papers which may prove useful to Shaw and Partners with regards to their product. This search produced 58 papers on the subject of antimicrobial effects of copper and cotton, this is too large a number for this report, so 6 potentially useful papers were described in this report. The above link will provide a search of all the relevant papers should Shaw and Partners wish to investigate further.

In situ antimicrobial behavior of materials with copper-based additives in a hospital environment (Palza *et al.*, 2018)

Link - https://www.sciencedirect.com/science/article/abs/pii/S0924857918300451?via%3Dihub

This paper reviewed multiple studies looking at the effectiveness of materials embedded with copper nanoparticles in a hospital environment. Plastic waiting room chairs with embedded metal copper nanoparticles, and metal hospital IV poles coated with an organic paint with nanostructured zeolite/copper particles were produced and tested in a hospital environment. These prototypes were sampled once weekly for 10 weeks, and the viable microorganisms were analysed and compared with the copper–free materials. In the waiting rooms, chairs with copper reduced by around 73% the total viable microorganisms present, showing activity regardless of the microorganism tested. The metal IV poles showed non-significant reductions in microorganisms, attributed to the rigorous cleaning protocols employed in operating theatres.

Antibacterial Cotton Fabric Functionalized with Copper Oxide Nanoparticles (Román *et al.*, 2020)

Link - https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7764683/

This review focusses on textiles functionalized with cupric oxide nanoparticles, which were classified into two groups, namely, in-situ (one step process) and ex-situ (two step process). The in-situ method consists of synthesizing nanoparticles in the presence of textile materials, whilst the ex-situ method, there are two steps, firstly synthesizing nanoparticles, then, in the second step, these nanoparticles are applied to textile materials. This paper discusses many examples of both methods but does not indicate which (if either) method is more effective, but suggests that in-situ methods may provide greater resistance to laundry cycles. The paper acknowledges that the method by which copper nanoparticles have an antimicrobial effect is poorly understood and discusses three possible mechanisms: the release of copper ions, the direct contact of CuO NPs with bacteria, and the production of reactive oxygen species.

Intrafibrillar Dispersion of Cuprous Oxide (Cu_2O) Nanoflowers within Cotton Cellulose Fabrics for Permanent Antibacterial, Antifungal and Antiviral Activity (Hillyer, Nam and Condon, 2022)

Link - https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9692297/

This paper from Hillyer *et al.* (Hillyer, Nam and Condon, 2022) describes a method of synthesis of Cu_2O nanoparticles, sized approximately 72 nanometres, that form within cotton fibres. These nanoparticles took a nanoflower morphology, providing an increased surface area. The presence of these nanoflowers within the fibres was confirmed using transmission electron microscopy.

The intrafibrillar nature of the nanoflowers meant they were resistant to washing away during laundry cycles, with only 19% being washed away after 50 cycles. The treated fabrics demonstrated broad-spectrum antibacterial, antifungal and antiviral activity with greater than 99.99% inhibition against K. pneumoniae, E. coli, S. aureus and A. niger and ≥90% inhibition against Human coronavirus, strain 229E, even after 50 home laundering cycles.

Manufacturing methodology is reported as:

> *"To 80 mL of DI water was dissolved 0.250 g cupric sulfate anhydrous. To the stirring solution of copper sulfate was slowly added 20 mL of 50% w/v*

sodium hydroxide solution to produce a 0.250 g·100 mL−1 stock solution. Upon addition of sodium hydroxide, the clear and light blue solution (CuSO4) initially precipitated a light blue solid (Cu(OH)2), which dissolved upon complete addition of the 20 mL of 50% w/v sodium hydroxide solution to become a clear dark blue solution ([Cu(OH)4]2−). Additional solutions were prepared from this 0.250 g·100 mL−1 stock solution by dilution with 10% w/v sodium hydroxide solution with concentrations 0.250 g·100 mL−1, 0.100 g·100 mL−1, 0.050 g·100 mL−1, 0.010 g·100 mL−1, 0.005 g·100 mL−1, 0.001 g/100 mL−1, and a control sample with only sodium hydroxide. A 50 mm × 200 mm swatch of bleached and desized cotton print cloth fabric was added to a 50 mL centrifuge tube and 40 mL of [Cu(OH)4]2− solution was added. The centrifuge tube was mixed at 750 rpm for 30 min using an Advanced Vortex Mixer (Fisher Scientific, Hampton, NH, USA). The fabric changed from a white to dark blue color. The fabric was then removed from the solution and rinsed with DI water to remove excess copper precursor and sodium hydroxide, changing from dark blue to light blue when the excess base was removed. The fabric was added to 80 °C DI water, where the light blue fabric changed immediately to dark brown. The fabric was removed, rinsed in DI water and air dried at room temperature."

Highly stable, antiviral, antibacterial cotton textiles via molecular engineering (Qian *et al.*, 2023)

Link - https://www.nature.com/articles/s41565-022-01278-y

This paper from Qian *et al.* 2023 (Qian *et al.*, 2023) details a method to fabricate antiviral and antibacterial cotton textiles which incorporate copper ions into the cotton structure at the atomic level, with ions of copper binding to the cellulose atoms that make up cotton. This atomic level attachment provided significant resistance to washing and air-based removal of the copper ions, the group estimated that it would take thousands of washes to remove half of the copper. The copper ion textile exhibited high antiviral and antibacterial activities against tobacco mosaic virus (TMV), influenza A virus (IAV), and Escherichia coli, Salmonella typhimurium, Pseudomonas aeruginosa and Bacillus subtilis bacteria.

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This paper also assessed the toxicity of the copper cotton hybrid using artificial perspiration on human cells. The results demonstrated that the copper ion textile does not cause cell death due to ions produced from the contact between the textile and human sweat. The group demonstrated the scalability of the method by producing a potential method for large amounts of treated cotton to be produced, as well as manufacturing an entire garment (t shirt) from the material. An additional finding, was that the tensile strength of the copper ion material was approximately 23% higher than that of the unmodified material, indicating that copper cotton hybrid materials are stiffer than cotton alone.

Manufacturing methodology is listed as:

> *"NaOH solutions of 5, 10, 20, and 40 wt% were prepared by dissolving NaOH (Sigma-Aldrich) in DI water. Cu(II)-saturated NaOH aqueous solution was prepared by immersing copper wires (Alpha Wire Company) in the NaOH solutions, keeping the preparation static until no further darkening of the blue colour was observed (typically in less than 2 days). After that, the cotton textiles were soaked in the Cu(II)-saturated NaOH solutions until they turned into stable blue colour (typically in 3 days). The Cu-IT samples were then washed with DI water several times until the pH value of the waste reached 7, and were then dried at room temperature."*

Antibacterial and Antimycotic Activity of Cotton Fabrics, Impregnated with Silver and Binary Silver/Copper Nanoparticles (Eremenko *et al.*, 2016)

Link - https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4717125/

This paper demonstrated a method of obtaining bactericidal bandage materials by impregnation of cotton fabric by aqueous solutions of silver and copper salts and a certain regime of heat treatment is developed. High antibacterial and antifungal properties of the obtained materials are confirmed in experiments with a wide range of multidrug-resistant bacteria and fungi.

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Silver and copper nanoparticles are known for their antibacterial properties against a wide spectrum of pathogenic and opportunistic bacteria. This paper utilised a much simpler method of producing cotton hybrid materials, by immersing in water solutions of silver or copper for 15 min, then squeezed thoroughly and evenly ironed at 200–220 °C by means of metallic press. Results showed that silver particles are sufficiently bound to the cotton fabric, which can retain good bacteriostatic properties even after washing. The study claimed a concentration of 0.015–0.13 percent by weight of copper or silver was sufficient to produce high antimicrobial properties against a wide range of multidrug-resistant bacteria Escherichia coli, Enterobacter aerogenes, Proteus mirabilis, K. pneumoniae, Candida albicans yeasts, and micromycetes, and activity remained high throughout 6 months.

Antibacterial Activity of Copper Particles Embedded in Knitted Fabrics (Ivanauskas *et al.*, 2022)

Link - https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9607619/

This paper demonstrates a simple chemical reduction method for the preparation of copper particles in knitted fabrics, which has excellent antibacterial properties against gram-positive Staphylococcus aureus and Escherichia coli bacteria.

Remigijus Ivanauskas and colleagues (2022) present an environmentally friendlier route for chemical synthesis of copper particles using copper(II) sulfate and L-ascorbic acid. The antibacterial efficacy of untreated wool and cotton knitted fabrics and those modified with copper particles fabrics was tested against Staphylococcus aureus and Escherichia coli. The bactericidal effect of metal and metal compound nanoparticles is attributed to their small size and high surface to volume ratio. They suggest that one of the possible uses for this fabric would be the production of long-term individual protective masks with inserts containing copper particles.

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They advocate that the synthesis of copper particles embedded in wool or cotton knitted fabrics and preparation of inserts for reusable face masks is discussed. The copper particles remained in the used knitted fabrics after washing with distilled water, while other products from the chemical reaction that formed the particles were removed.

The antibacterial efficacy of untreated wool and cotton knitted fabrics and those modified with copper particles fabrics was tested against gram-positive bacteria Staphylococcus aureus and gram-negative bacteria Escherichia coli. Untreated fabrics showed no antimicrobial activity, whereas treated cotton showed good antimicrobial activity, whereas treated wool performed even better.

Manufacture methodology described as:

> *"In a two-stage synthesis, particles containing copper were synthesized by a simple reduction method over the entire textile volume. In the first stage, a sample of wool (W) or cotton (C) knitted fabric was saturated in 0.5 mol/L solution of copper(II) sulfate at 25 °C, and in the second stage it was treated with a 0.6 mol/L solution of reducing agent (ascorbic acid) at 40 °C for 720 min. The formed copper particles remained in the used knitted fabrics after washing with distilled water, while other reaction products were removed. Then, the samples modified with copper particles were dried and used in further studies. The synthesis of copper particles embedded in wool or cotton knitted fabrics and the preparation of inserts for reusable face masks are shown in Figure 1."*

3.2. Potential testing method for copper cotton hybrid materials

Here we will briefly describe a testing protocol for assessing the antimicrobial activity of copper cotton hybrid materials (CCHMs). This is a crude test and is designed to provide a preliminary idea of the antibacterial properties of the material.

The core concept of this test is to assess whether a CCHM is capable of killing more bacteria than an untreated, non-hybrid cotton material. To do this, similar samples of clean CCHM and untreated cotton must be obtained, contaminated with bacteria (in this case, harmless Lactobacillus from live yogurt), left for 5 minutes (shorter or longer if necessary), then the two cotton samples must be assessed to see how many bacteria still survive. This final step is typically performed by giving any surviving bacteria perfect conditions to grow, which is done by pressing an agar contact plate onto the surface, which will pick up any surviving bacteria, and incubating the plate for 24 hours at 37 °C. Each bacterium that survives will rapidly multiply, forming a colony of bacteria on the agar contact plate, the more colonies that are present after 24 hours indicate that more bacteria survived contact with the material, therefore it is less antibacterial.

Instructions:

1. Mix 5 mL of live yogurt with 500 mL of fresh water and place into a spray bottle
2. Spray this bacterial solution onto the two materials
3. Leave for 5 minutes to allow the antimicrobial properties of the treated material to take effect
4. Press one contact agar plate onto each material and seal - https://www.sigmaaldrich.com/GB/en/product/mm/146552
5. Incubate the contact plate for 24 hours at 37 °C
6. Count the colonies that have formed on the plate that tested each material
7. If the CCHM has antibacterial properties, there should be far fewer colonies on the CCHM contact plate
8. If colony numbers are too high, or too low, then the time period at point 3 is likely too short or too long respectively, repeat the experiment with altered time frames

4. Conclusion

This report summarises a number of scientific investigations into the functionality of copper cotton hybrid materials (CCHMs). Relatively few reviews regarding this subject were found, suggesting that this is a poorly explored field in scientific literature. The 2018 review by Palza et al illustrated the value of these hybrid materials in the clinical setting, with a 73% reduction in microorganisms on chairs treated with copper nanoparticles. A review of the different methods of CCHM manufacture suggests that making and embedding copper nanoparticles into cotton in a single step may make it more resistant to laundering cycles, but does not indicate which has a better antimicrobial ability. In addition, the report indicates that the method of antimicrobial action of copper nanoparticles is still poorly understood.

Hillyer's 2022 research showed an inhibitiory effect of up to 99.99% against multiple harmful pathogens, even after 50 home laundering cycles, with 81% of the embedded nanoparticles remaining after these cycles. Research from Qian et al. supported this, estimating that it would take thousands of washes to reduce the antimicrobial activity by 50%, and that the treated fabrics were not toxic to cells grown in culture. A further paper from Eremenko et al. suggested a concentration of 0.015-0.13% copper by weight was enough to produce significant antimicrobial properties for a period of 6 months.

5. Bibliography

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