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Potent Antibacterial Activity of Copper Embedded into Silicone and Polyurethane

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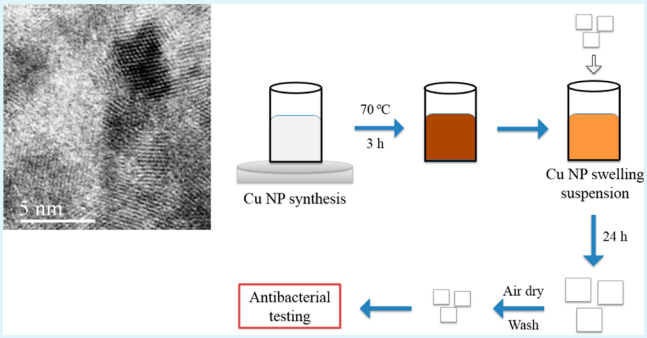
Supporting Information

ABSTRACT: A simple, easily up-scalable swell−encapsulation−shrink technique was used to incorporate small 2.5 nm copper nanoparticles (CuNPs) into two widely used medical grade polymers, polyurethane, and silicone, with no significant impact on polymer coloration. Both medical grade polymers with incorporated CuNPs demonstrated potent antimicrobial activity against the clinically relevant bacteria, methicillin-resistant *Staphylococcus aureus* and *Escherichia coli*. CuNP-incorporated silicone samples displayed potent antibacterial activity against both bacteria within 6 h. CuNP-incorporated polyurethane exhibited more efficacious antimicrobial activity, resulting in a 99.9% reduction in the numbers of both bacteria within just 2 h. With the high prevalence of hospital-acquired infections, the use of antimicrobial materials such as these CuNP-incorporated polymers could contribute to reducing microbial contamination associated with frequently touched surfaces in and around hospital wards (e.g., bed rails, overbed tables, push plates, etc.).



KEYWORDS: *antimicrobial, polymers, nanoparticle, hospital-acquired infections, bacteria, copper*

INTRODUCTION

Hospital acquired infections (HAIs) are the most common complication in healthcare, contributing toward morbidity, mortality, and increased medical costs.[1] They can occur in various care environments, such as same-day surgical centers, short-term care within hospitals ,and long-term care facilities (e.g., nursing homes).[2] Over the last century, medicine has advanced, and new antibiotics have been introduced. Consequently, bacteria have rapidly evolved resistance mechanisms, and this has led to an increasing threat to patients posed by antibiotic-resistant "superbugs".[3] Moreover, in addition to relieving suffering, preventing HAIs is financially beneficial, with savings of billions of pounds to health service in the United Kingdom alone.[4] Around 75% of patient rooms are contaminated with methicillin-resistant *Staphylococcus aureus* (MRSA), and 42% of people who touch a contaminated surface become contaminated with MRSA themselves, despite not having direct patient contact.[4] Carbapenem-resistant Enterobacteriaceae (CRE) are another major problem associated with HAIs, especially for immunocompromised and catheterized patients, and treatment options are extremely limited.[5,6]

Modern healthcare uses different types of invasive devices to treat patients, and these are often associated with an increased risk of infection, including catheter-associated urinary tract infections and ventilator-associated pneumonia.[7] Our research group has developed new ways to potentially reduce HAIs associated with medical devices, by developing new polymeric materials that can be used as catheter materials and to coat hospital surfaces.[8−17] Such materials include silicone rubber, polyvinyl chloride, polyurethane and polypropylene.[18] These materials can also be used to treat HAIs caused by commonly

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touched surfaces in hospital wards (e.g., bed rails, overbed tables, call buttons).

Some metal nanoparticles are known to have bactericidal effects due to their high surface-to-volume ratio and small size which allows them to closely interact with bacterial cell membranes,[19] rather than the release of metal ions into solution.[20] They can therefore be used in applications such as water treatment, food processing and medical devices.[21] Copper nanoparticles in particular, are crucial in many applications, such as catalysts and optical devices,[22] but they are also known for their excellent antimicrobial properties. This could be due to an increased concentration of copper inside the cell, leading to the Fenton Process which causes oxidative stress and forms hydrogen peroxide.[23] Furthermore, excess copper causes a decrease of the membrane integrity of microorganisms, leading to the loss of vital nutritional cell elements, causing desiccation and eventually cell death.[24]

Despite this, there are very few reports on the preparation of copper nanoparticles using a route that has a low environmental impact commonly known as a "green method". It is imperative that for use in healthcare applications, a green synthetic route using nontoxic chemicals, environmentally friendly solvents and renewable materials is developed.[25] We have adapted a method for synthesizing environmentally benign 2.5 nm copper nanoparticles and subsequently encapsulated them into two key medical grade polymers for healthcare applications, polyurethane and silicone.

In this study, we investigate the antibacterial activity of two widely used polymers, medical grade silicone and polyurethane, when encapsulated with copper nanoparticles. Cu-polyurethane and Cu-silicone were tested against a model Gram-negative bacterium, *Escherichia coli*, and an epidemic strain of MRSA, EMRSA-16 as a representative Gram-positive bacterium. Cu-polyurethane displayed the most potent kill against both bacteria, with a reduction in the numbers of MRSA to below the detection limit after only 2 h of contact and after 3 h in the case of *E. coli*. Cu-silicone also displayed bactericidal activity against *E. coli* and MRSA, though less than Cu-polyurethane; however, it remained completely colorless after the swell–encapsulation process, proving commercially appealing. This is, to the best of our knowledge, the first example of CuNPs encapsulated into polymers for antibacterial activity.

EXPERIMENTAL SECTION

Materials and Methods. *Chemicals and Reagents.* All reagents used in the synthesis of copper nanoparticles (CuNPs) were commercially supplied by Sigma-Aldrich Chemical Co.: $CuCl_2{\cdot}2H_2O$ acted as the precursor for nanoparticle formation and L-ascorbic acid acted as the reducing agent and capping agent. Polymer substrates used were medical grade flat polyurethane sheets (thickness 0.8 mm) purchased from American Polyfilm Inc. (Branford, CT) and medical grade flat silicone sheets (thickness 1.0 mm) purchased from NuSil (Polymer Systems Technology, Ltd.). Deionized water (resistivity 15 MΩ cm) was used throughout the experiments. Acetone (Sigma-Aldrich, U.K.) was used to aid the swell-encapsulation process.

Synthesis of CuNPs. The copper nanoparticles were prepared using a method based on that described by Xiong et al.[26] An aqueous solution of $CuCl_2{\cdot}2H_2O$ (0.2 M) was heated to 70 °C with constant stirring, after which L-ascorbic acid aqueous solution (0.6 M) was added dropwise. The reaction vessel was sealed and the temperature was maintained at 70 °C for 3 h until a dark solution was obtained.

Material Preparation. Copper-encapsulated polyurethane and silicone polymer samples were prepared using a simple swell–encapsulation–shrink procedure. Polymer squares (1 cm^2) were immersed in a 9:1 acetone/CuNP swelling solution for 24 h, after which they were removed from solution, air-dried overnight, and washed with distilled water (Figure S1 in Supporting Information).

Material Characterization. CuNPs suspended in aqueous solution were drop-cast onto a 400 Cu mesh lacey carbon film TEM grid (Agar Scientific Ltd.) and imaged using a Jeol 2100 high-resolution transmission electron microscope (HR-TEM) with a LaB_6 source operating at an acceleration voltage of 200 kV with an Oxford Instruments XMax EDS detector running AZTEC software. The images were analyzed using ImageJ software and energy-dispersive X-ray (EDX) spectra obtained. Scanning electron microscopy (SEM) of polyurethane, silicone, and CuNP incorporated polyurethane and silicone polymers was performed using secondary electron imaging on a JEOL 6301 field emission instrument with an acceleration voltage of 5 kV.

UV–vis absorption spectra of the polymer samples were recorded using a PerkinElmer Fourier transform Lambda 950 UV–vis spectrometer (350–550 nm range). X-ray photoelectron spectroscopy (XPS) analysis was performed using a Thermo Scientific K-Alpha spectrometer to detect copper as a function of polymer depth. All binding energies were calibrated to the C 1s peak at 284.5 eV. To determine the difference in surface hydrophobicity of the treated polymer samples, we obtained equilibrium water contact angle measurements (~5.0 μL) using an FTA 1000 Drop Shape Instrument and analyzed using FTA32 software. The contact angle measurements for each sample type were taken to be the average value of ≥10 measurements using a droplet of deionized water dispensed by gravity from a 30 gauge needle.

Antibacterial Activity. *Microbiological Method.* The following 1 cm^2 polyurethane and silicone samples were used in the microbiology experiments: (1) solvent treated (control) and (2) copper nanoparticle-encapsulated polymer. The antibacterial activity of these samples was tested against EMRSA-16 and *E. coli* ATCC 25922. These organisms were stored at −70 °C in Brain–Heart Infusion broth (BHI, Oxoid) containing 20% (v/v) glycerol and propagated onto either Mannitol salt agar (MSA, Oxoid) in the case of MRSA or MacConkey agar (MAC, Oxoid) in the case of *E. coli*, for a maximum of 2 subcultures at intervals of 2 weeks.

BHI broth was inoculated with 1 bacterial colony and cultured in air at 37 °C for 18 h with shaking, at 200 rpm. The bacterial pellet was recovered by centrifugation, (20 °C, 2867.2*g*, 5 min), washed in PBS (10 mL), and centrifuged again to recover the pellet (20 °C, 2867.2*g*, 5 min), and the bacteria were finally resuspended in PBS (10 mL). The washed suspension was diluted 1000-fold to obtain an inoculum of ~10^6 cfu/mL. In each experiment, the inoculum was confirmed by plating 10-fold serial dilutions on agar for viable counts. Triplicates of each polymer sample type were inoculated with 25 μL of the inoculum and covered with a sterile coverslip (2.2 cm^2). The samples were then incubated in the dark for up to 6 h for the silicone samples and 3 h for the polyurethane samples.

After incubation, the inoculated samples and coverslips were added to PBS (450 μL) and mixed using a vortex mixer. The neat suspension and 10-fold serial dilutions were plated on agar for viable counts and incubated aerobically at 37 °C for 24 (*E. coli*) or 48 h (MRSA). The experiment was repeated three times and the statistical significance of the following comparisons was analyzed using the Mann–Whitney U test: (1) control (polymer only) vs inoculum; (2) copper nanoparticle vs control. To examine the stability of the modified polymer squares, their antibacterial activity was retested against both bacteria after 30 days and 90 days storage at room temperature in air.

Bovine serum albumin (BSA, 0.03%) and superoxide dismutase (SOD, 55 U mL^{-1}; Sigma-Aldrich, United Kingdom) were added to the *E. coli* suspension and exposed to both polymers as described in the protocol above. They were filter sterilized using a 0.2 μm syringe filter (VWR, United Kingdom). BSA was added to simulate contamination in a hospital setting with organic material, as well as acting as a scavenger of reactive oxygen species (particularly singlet oxygen), and SOD was added as an inhibitor of superoxide.

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RESULTS AND DISCUSSION

Characterization of CuNPs. A green route was used to synthesize the copper nanoparticles by dropwise addition of L-ascorbic acid to a gently heated aqueous solution of copper(II) chloride. The solution was further heated, and reaction completion was indicated when a dark orange solution formed. HR-TEM images of CuNPs in solution showed the particles to be mainly spherical, nanocrystalline and monodisperse (Figure 1a). Although some nanoparticle agglomeration was observed,

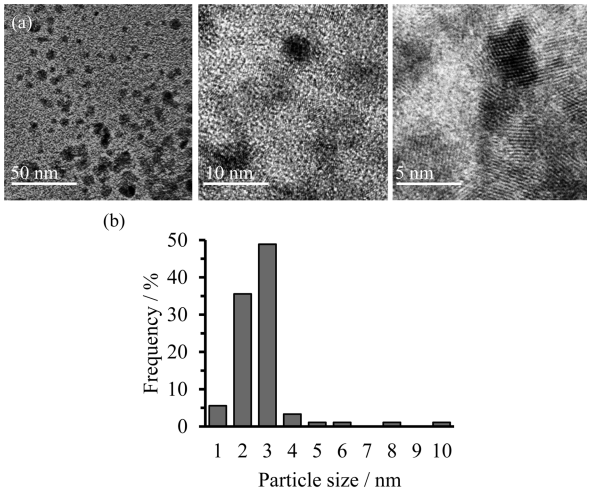

Figure 1. (a) TEM images showing CuNPs. (b) Particle size distribution of CuNPs, determined by TEM.

with evidence of clusters ~10 nm in diameter, overall, size analysis showed an average nanoparticle diameter of 2.5 ± 0.7 nm with a fairly narrow size distribution and uniformity in shape (Figure 1b). Narrow size control was achieved by using the antioxidant, L-ascorbic acid, which was effective in reducing Cu^{2+} to Cu^0 and capping the CuNPs, such that the resultant small nanoparticles were stable to oxidation. EDX elemental composition analysis (Figure S2, Supporting Information) confirmed a strong presence of Cu, with limited evidence for CuNP oxidation. SEM imaging indicated no physical change in the surface properties of the polymers upon incorporation of the CuNPs (Figure S3, Supporting Information).

Characterization of Polymer Samples. To introduce antimicrobial functionality into commonly used polymers for medical device applications such as polyurethane and silicone, we used a simple swell−encapsulation−shrink method for the incorporation of small CuNPs. The commercially available polymers were swelled in a 9:1 acetone/aqueous CuNP solution for 24 h, achieving diffusion of nanoparticles throughout the polymer bulk.[27] As shown in Figure 2, one advantage of achieving antimicrobial functionality through encapsulation of these nanoparticles is a limited change in polymer coloration upon their incorporation. This is particularly important where aesthetics is of interest, for

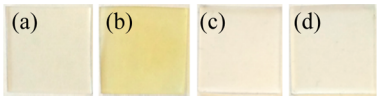

Figure 2. Polymer squares (1 cm^2) of all samples tested for antimicrobial activity: (a) polyurethane control; (b) Cu-encapsulated polyurethane; (c) silicone control; (d) Cu-encapsulated silicone.

example in commercial applications for frequently touched surfaces in hospitals. It should be noted that the slight coloration of the polyurethane sample may be attributed to significantly greater polymer swelling under the conditions used, resulting in an increased uptake of the CuNPs. However, this is advantageous in antimicrobial applications, where a comparatively enhanced antimicrobial efficacy is anticipated.

UV−vis absorbance spectroscopy measurements demonstrated greater absorbance signals for Cu-incorporated polymers compared to control polymer samples, providing evidence for CuNP uptake (Figure S4, Supporting Information), and a higher uptake of CuNP in polyurethane was noted, compared to that achieved in silicone. Swelling measurements were also carried out and confirmed that polyurethane swelled up 50% more than its original size, whereas silicone only swelled 30% greater than its original size. Thus, CuNP-incorporated polyurethane had a slight coloration as it can be suggested that a higher concentration of CuNPs are encapsulated into the polymer.

The CuNP-incorporated silicone and polyurethane samples were analyzed using XPS to examine the diffusion of CuNP through the polymer surface, achieved using the swell−encapsulation−shrink synthetic strategy. XPS was used to analyze the presence of copper on the surface and within the polymer bulk (sputtered 50 s). For all the polymer samples, peaks attributed to the presence of C (1s), N (1s), and O (1s) on the surface were observed (data not shown). XPS analysis of the CuNP-embedded polyurethane sample showed evidence of copper, confirming the presence of CuNPs both at the polymer surface and encapsulated within the polymer matrix. As shown in Figure 3a,b, a peak in the Cu (2p) correlating to copper was observed, confirming the presence of Cu on the polyurethane surface and encapsulated within the polymer bulk (952.3 eV). A peak in the Cu (2p) region correlating to Cu in CuO (932.4 eV) is also evident, indicating some oxidation of the surface encapsulated CuNP. Interestingly, the silicone samples showed limited copper at the polymer surface (Figure S5, Supporting Information), with significant CuNP encapsulation in the polymer bulk indicated by peaks in the Cu (2p) region for Cu in CuO and Cu metal at 933.1 and 952.3 eV, respectively (Figure 3c). Overall XPS depth profile data for both silicone and polyurethane polymers encapsulated with CuNPs showed a higher copper content within the polymer bulk compared to at the surface.

The wetting properties of the polyurethane and silicone samples used for microbiological testing were investigated under standard laboratory conditions. The water contact angle measurements indicated that polyurethane and silicone polymer controls present a hydrophobic surface, with a slight increase in hydrophobicity observed upon incorporation of the CuNPs (Table. 1). Polyurethane demonstrated a larger water contact angle difference (increase of ~15.8°) after swell-encapsulation, compared to silicone (increase of ~4.8°). It can be suggested that because the polyurethane polymer swells more, it can achieve a higher concentration of encapsulated CuNPs, modifying the surface properties to a greater degree. This is important for biofilm formation, as hydrophobic surfaces prevent bacteria from adhering to the polymer surface.[10]

Antimicrobial Investigation. The antibacterial activity of a range of CuNP modified polymer samples was tested against representative Gram-positive and Gram-negative bacteria, which are known to be important pathogens in HAIs.[28] Figure

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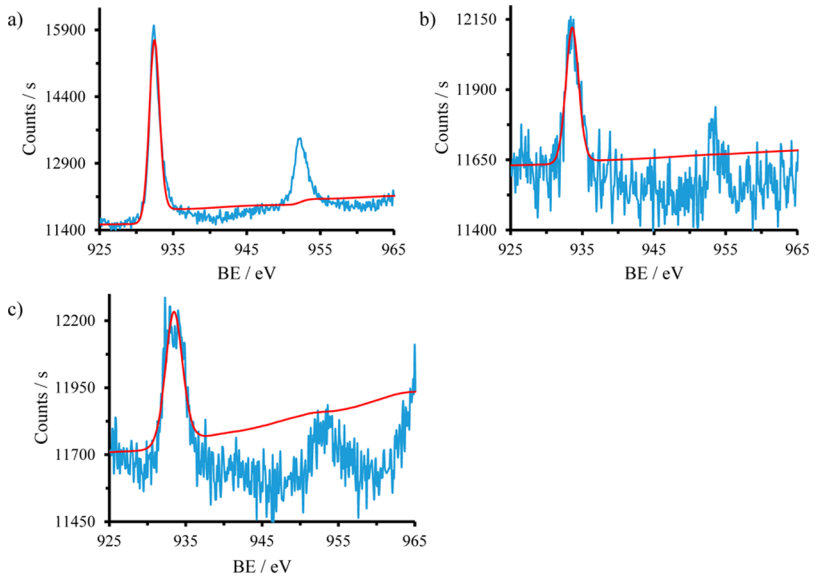

Figure 3. Copper 2p region XPS spectra for (a) Cu-polyurethane surface; (b) Cu-polyurethane sputtered 50 s; (c) Cu-silicone sputtered 50 s.

Table 1. Average Contact Angle Measurements of Water and Standard Deviation on Polyurethane Control, Cu-Encapsulated Polyurethane, Silicone Control and Cu-Encapsulated Silicone

polymer sample	contact angle (deg)	std deviation
polyurethane	98.5	± 1.1
Cu−polyurethane	114.3	± 2.0
silicone	115.0	± 2.5
Cu−silicone	119.8	± 1.4

4 illustrates the bactericidal activity against EMRSA-16 of polyurethane (control), Cu-encapsulated polyurethane, silicone (control), and Cu-encapsulated silicone. After 1 h of incubation (Figure 4a), the control polyurethane sample did not show significant kill of EMRSA-16, whereas Cu-polyurethane exhibited a ∼ 0.75 log reduction in bacterial numbers (P < 0.001). However, by increasing the exposure time to 2 h (Figure 4b), Cu-polyurethane demonstrated highly significant bactericidal activity (≥4 log reduction in the bacterial numbers; P < 0.001). The clinical strain of MRSA was exposed to silicone and Cu-silicone for longer periods of time (Figure 4c,d). After 4 h of incubation, the control silicone did not display significant kill of EMRSA-16, but Cu-silicone caused a 1 log reduction in bacterial numbers. By increasing the time to 5 h, Cu-silicone

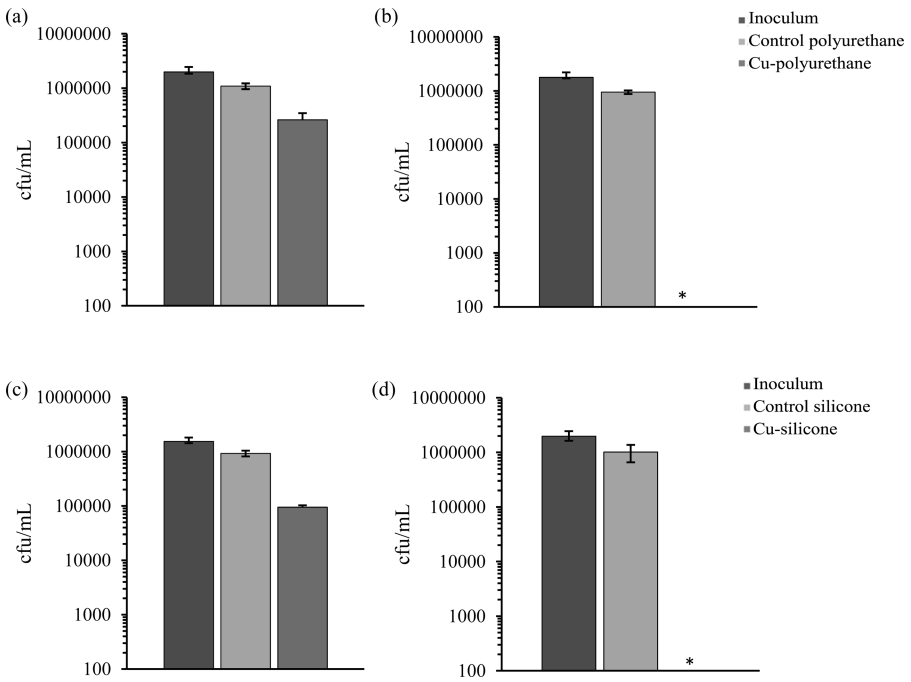

Figure 4. Viable counts of EMRSA-16 after incubation on modified polyurethane squares for: (a) 1 and (b) 2 h, and modified silicone squares for: (c) 4 and (d) 5 h. All the samples were incubated at 20 °C in the dark. Control samples are solvent treated; * indicates bacterial numbers reduced below the detection limit of 100 cfu/mL.

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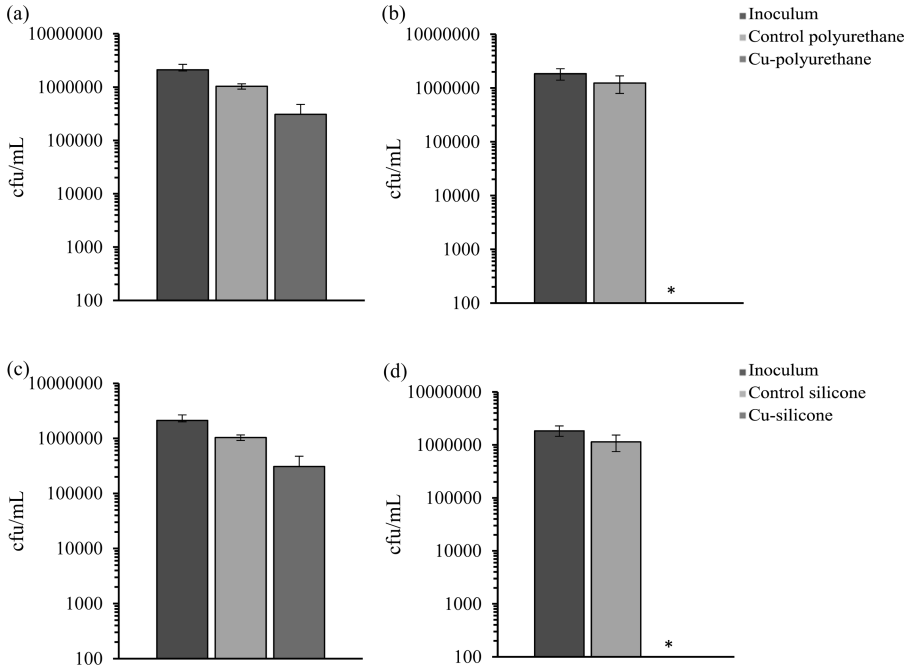

Figure 5. Viable counts of *E. coli* ATCC 25922 after incubation on modified polyurethane squares for: (a) 2 and (b) 3 h, and modified silicone squares for: (c) 4 and (d) 6 h. All samples were incubated at 20 °C in the dark. Control samples are solvent treated; * indicates bacterial numbers reduced below the detection limit of 100 cfu/mL.

resulted in the greatest kill, with bacterial numbers reduced to below the detection limit of 100 cfu/mL (≥4 log; $P < 0.001$).

The antibacterial activity of the same polymer samples was tested against the Gram-negative bacterium, *E. coli*, under the same conditions but for an extended period of time (Figure 5). Figure 5a shows the activity of the samples following 2 h of bacterial contact, where polyurethane displayed no significant activity, however, Cu-polyurethane resulted in a ∼ 0.5 log reduction in bacterial numbers. After 3 h, Cu-polyurethane showed highly significant bactericidal activity against *E. coli* ($P < 0.001$), reducing bacterial numbers by ≥4 log. Figure 5c illustrates the antibacterial activity of silicone and Cu-silicone after 4 h of contact with *E. coli*. Whereas no significant decrease in the numbers of bacteria was recorded with silicone alone, Cu-silicone gave ∼0.5 log reduction in bacterial numbers and after 5 h of incubation in the dark, Cu-silicone achieved $a \geq 4$ log reduction in the numbers of *E. coli* ($P < 0.001$).

Using the swell−encapsulation−shrink strategy, the polymers were exposed to L-ascorbic acid concentrations as described in the material preparation section. Testing of these polymer samples against both *E. coli* and EMRSA-16 indicated that the unbound L-ascorbic acid alone, does not demonstrate antibacterial activity (data not shown).

Xiong et al. examined the stability of their copper solution and reported that it was stable even after storage for 2 months.[26] In this study, the stability of the copper nanoparticles after encapsulation into the polymer was investigated, by assessing the bactericidal activity of the samples 30 days and 90 days after they were prepared. Even after 90 days, the samples remained active, giving similar levels of bacterial kill as reported for the fresh polymers in Figures 4 and 5 (data not shown). The nanoparticles were successfully encapsulated into the polymer via a simple swell−encapsulation−shrink method which is a much easier and quicker route than other routes toward the incorporation of NPs within polymeric materials, such as covalent attachment to the polymer surface.[27] Furthermore, the encapsulation of nanoparticles within the polymer substrate reduces nanoparticle loss by washing or wiping the surface.

It can be speculated that CuNPs leach from the polymer into the surrounding bacterial solution, resulting in the potent bactericidal activity demonstrated with kills achieved via a combination of various mechanisms. A high concentration of copper ions leaching into the solution could be producing reactive oxygen species causing progressive oxidative damage, resulting in cell death.[29] Additionally, the interaction between the bacterial outer membrane and copper surface can cause the membrane to rupture, or cause holes in the outer membrane, which can weaken the cell via the loss of vital nutrients and water.[30]

To gain an understanding of the mechanism operating within this system, we included the effect of a superoxide inhibitor (SOD, 55 U mL^{-1}) in the antibacterial tests against *E. coli*.[31] We did not see any significant change in antibacterial activity, implying that superoxide radicals were not responsible for the potent activity observed from the CuNPs (data not shown). With addition of BSA included in the protocol (but without SOD), we also did not see any significant change in the antibacterial activity of the samples against *E. coli*, which indicates resilience to contamination that could occur from organic materials contained in a hospital environment (data not shown). This result contrasts with other experiments conducted by our research group using photoactivatable polymer surfaces where we have observed a significant reduction in bacterial kill with the addition of BSA to the system, although this was ascribed to scavenging of reactive oxygen species.[17]

The average light intensity in hospitals is reported to range between 1000 lx in an accident and emergency examination room to 10 000−100 000 lx in an operating theater.[29] However, actual measurements in some hospital wards are as low as 200 lx (Dr P. Wilson, UCLH, personal communica-

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tion). By demonstrating antibacterial activity in the dark, we eliminate the concern associated with variable light intensities of different areas in a hospital building. Our previous work has largely required light to activate samples as they have been incorporated with photosensitizers such as crystal violet and methylene blue to kill bacteria for a nonsite-specific bacterial attack. Certain nanoparticles (Au, ZnO, MgO) have also been combined with photosensitizers to enhance their effect.[9,10,12−17,29,30]

In this paper, we have achieved highly significant antibacterial activity without the need for light and without effecting a change in the appearance of the materials. For aesthetic reasons, a material that is not brightly colored yet rapidly kills bacteria is more appealing for frequently touched surfaces in and around hospital wards. Even though we achieve a much faster kill with polyurethane compared to silicone, it does undergo a slight change in polymer coloration upon incorporation of CuNPs, whereas no visible change was noted for the silicone polymer after nanoparticle incorporation. However, as the polyurethane swells more than silicone, a greater concentration of CuNPs is incorporated into the polymer. In addition to this, we see a greater increase in hydrophobicity of polyurethane after swell-encapsulation, resulting in a greater bactericidal effect of Cu-polyurethane than Cu-silicone against both bacteria tested.

Furthermore, we have effectively synthesized nontoxic, cost-effective 2.5 nm copper nanoparticles using a straightforward method that is easily reproducible. These nanoparticles have been incorporated into two polymer types that are commonly used in hospitals for medical devices and coating surfaces such as keyboards, mousepads, and other electronic device covers. We have achieved potent kill using a simple swell−encapsulation−shrink method to incorporate these nanoparticles and have shown them to be highly stable in both polymers for up to 90 days. These samples have the potential to help combat the current problem posed by multidrug resistant bacteria in the healthcare industry worldwide.

CONCLUSION

A green strategy was used to synthesize monodisperse CuNPs with a narrow size distribution (2.5 ± 0.7 nm in size). The CuNPs were synthesized using nontoxic reagents, highlighting their suitability for biomedical applications. A simple, easy to upscale swell−encapsulation−shrink strategy was used to incorporate the CuNPs into medical grade silicone and polyurethane polymers commonly used for healthcare applications and the resultant modified polymers demonstrated limited or no discoloration. The CuNP-incorporated polymers exhibited potent antimicrobial activity against an epidemic strain of MRSA, in addition to *E. coli*, both significant causes of HAIs. Within just 2 h, CuNP-incorporated polyurethane reduced the numbers of bacteria by 99.9% when tested against EMRSA-16 and a 99.9% reduction in the numbers of *E. coli* was achieved within 3 h. CuNP-incorporated silicone also demonstrated efficacious antimicrobial activity reducing the numbers of EMRSA-16 and *E. coli* to below the detection limit within 5 and 6 h, respectively. Upon the addition of organic material to replicate environmental contamination, the antibacterial activity of the samples did not change.

These novel, highly effective antimicrobial materials are commercially attractive and easy to synthesize and demonstrate potent antimicrobial activity against both Gram-positive and Gram-negative bacteria, showing strong potential use in hospitals for reducing the incidence of HAIs.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsami.5b08665.

Schematic diagram of material preparation, EDX spectrum of CuNPs, SEM imaging and UV−vis absorbance spectra of samples, and Copper 2p region XPS spectra for Cu−silicone surface. (PDF)

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Notes

The authors declare no competing financial interest.

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