Development of Antimicrobial Fabrics Based on Silver and Copper Nanoparticles

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ABSTRACT

With growing public unease surrounding the extent of microbial infections, there is a demand for antimicrobial materials including antimicrobial textiles. Nanotechnology has provided new solutions for the development of antimicrobial fabrics. In this study, nanoparticles of silver (Ag) and copper (Cu) and alloy nanoparticles of Ag and Cu (Ag/Cu) have been synthesized by reduction of their respective nitrates by ascorbic acid, using chitosan as a stabilising agent and microwave heating. UV-vis spectrophotometry indicated the presence of the alloy by a single peak (500 nm) for Ag/Cu nanoparticles, whereas mixtures of Ag and Cu nanoparticles (Ag+Cu) showed two peaks of 420 and 500 nm, corresponding to pure Ag and Cu nanoparticles respectively. Particle size is increased by increasing nitrate concentration and reducing the chitosan concentration. Surface zeta potentials were positive for all the nanoparticles and varied from +27.8 to +33.8 mV. Ag and Cu nanoparticles were shown to be spherical whilst the alloy nanoparticles had an irregular shape. Cu nanoparticles resulted in higher inactivation of bacteria such as Bacillus subtilis (B. subtilis), Escherichia coli (E. coli) and methicillin-resistant Staphylococcus aureus (MRSA) than did Ag nanoparticles at the same concentration. The effect was reversed when tested on nanoparticles of the same mean particle size with Ag nanoparticles emerging as more effective. Bacterial inactivation increased with concentration of chitosan and the metal concentration. The nanoparticles showed a more potent antibacterial effect than did ions of the same metal. B. subtilis was more susceptible than E. coli which may be due to the differences in their cell walls structure. MRSA proved harder to inactivate than both B. subtilis and E. coli under identical conditions. Antifungal activity was significantly affected by the types of nanoparticles employed. Ag nanoparticles displayed higher inactivation than Cu ones. Alloved nanoparticles demonstrated the highest inactivation against both bacteria and fungi. This constitutes clear evidence of an antimicrobial synergy between the Ag and Cu. Bacteria and fungi in contact with nanoparticle-impregnated fabrics were revealed by FEGSEM to have taken on a shrunken appearance. Nanoparticle-impregnated fabrics reduced microbial viability by 80-90%, but this decreased in relation to the number of washes the fabric was subjected to and indicated a leached out of the nanoparticles. Pre-treatment of cotton fabrics with tannic acid and citric acid enhanced the durability of the antimicrobial effect when washed and this increased with concentration of the acid. Citric acid treated fabrics showed higher durability than tannic acid treated fabrics. Log reductions of Trichophyton interdigitale (T. interdigitale) were lower than those for B. subtilis, E. coli and MRSA at the same test conditions. The combination of nanoparticles with the antifungal drug fluconazole proved effective and reduced the time necessary to eliminate the T. interdigitale than either nanoparticles or fluconazole alone.
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<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>ATR</td>
<td>Attenuated total reflectance</td>
</tr>
<tr>
<td>ATCC</td>
<td>American type culture collection</td>
</tr>
<tr>
<td>CFU</td>
<td>Colony forming unit</td>
</tr>
<tr>
<td>EDX</td>
<td>Energy dispersive X-ray</td>
</tr>
<tr>
<td>FEGSEM</td>
<td>Field emission gun scanning electron microscopy</td>
</tr>
<tr>
<td>FTIR</td>
<td>Fourier transform infrared spectroscopy</td>
</tr>
<tr>
<td>ICU</td>
<td>Intensive care unit</td>
</tr>
<tr>
<td>MBC</td>
<td>Minimum bactericidal concentration</td>
</tr>
<tr>
<td>MIC</td>
<td>Minimum inhibitory concentration</td>
</tr>
<tr>
<td>MFC</td>
<td>Minimum fungicidal concentration</td>
</tr>
<tr>
<td>MRSA</td>
<td>Methicillin-resistant <em>Staphylococcus aureus</em></td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffer saline</td>
</tr>
<tr>
<td>PHMB</td>
<td>Polyhexamethylene biguanide</td>
</tr>
<tr>
<td>QAC</td>
<td>Quaternary ammonium compound</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning electron microscopy</td>
</tr>
<tr>
<td>TEM</td>
<td>Transmission electron microscopy</td>
</tr>
<tr>
<td>TSA</td>
<td>Tryptone soya agar</td>
</tr>
<tr>
<td>TSB</td>
<td>Tryptone soya broth</td>
</tr>
<tr>
<td>UV-vis</td>
<td>Ultraviolet visible</td>
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## NOMENCLATURE

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>$N_0$</td>
<td>Initial number of bacteria</td>
</tr>
<tr>
<td>$N$</td>
<td>Number of bacteria after incubation</td>
</tr>
<tr>
<td>owf</td>
<td>On weight fibre</td>
</tr>
<tr>
<td>$P$</td>
<td>Probability</td>
</tr>
<tr>
<td>rpm</td>
<td>Revolutions per minute</td>
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<tr>
<td>$s$</td>
<td>Second</td>
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<tr>
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<td>$V$</td>
<td>Volt</td>
</tr>
<tr>
<td>$W$</td>
<td>Weight</td>
</tr>
<tr>
<td>$\times$</td>
<td>Magnification</td>
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Figure 6.8: Log reduction of Ag, Cu, Ag+Cu and Ag/Cu nanoparticles of same mean particle size of 200 nm impregnated onto fabrics against $10^8$ CFU/mL MRSA after 30 minutes contact time. Error bars represent the standard deviation from the mean of triplicate determinations. Different letters signify that the results are statistically significant ($p < 0.05$).

Figure 6.9: pH of tannic acid solutions at different concentrations. Data are the mean of triplicate determinations. Different letters signify that the results are statistically significant ($p < 0.05$).

Figure 6.10: FTIR-ATR of untreated and tannic acid treated cotton fabrics.

Figure 6.11: FTIR-ATR of untreated and citric acid treated cotton fabrics.

Figure 6.12: FTIR-ATR of untreated and citric acid treated cotton fabrics after curing process.

Figure 6.13: Schematic diagram of the pre-treatment of cotton fabric by tannic acid.

Figure 6.14: Schematic diagrams of pre-treatment of cotton fabric by citric acid (a) before and (b) after crosslinking.

Figure 7.1: Zone of inhibition of 50 mM Ag and Cu nanoparticles synthesized in 3 wt% chitosan and 50 mM ion-impregnated cotton fabrics against $10^6$ CFU/mL T. interdigitale after 7 days of incubation. Error bars represent the standard deviation from the mean of triplicate determinations. Different letters signify that the results are statistically significant ($p < 0.05$).
Figure 7.2: Zone of inhibition of impregnated cotton fabrics impregnated with Ag, Cu, Ag/Cu and Ag+Cu nanoparticles (a) synthesised in 50 mM metal salts and 3% (w/v) of chitosan (b) of same mean particle size of 200 nm impregnated into fabrics against $10^6$ CFU/mL *T. interdigitale* after 7 days of incubation. Error bars represent the standard deviation from the mean of triplicate determinations. Different letters signify that the results are statistically significant ($p < 0.05$).

Figure 7.3: Log reduction of Ag and Cu nanoparticle- and ion-impregnated cotton fabrics against $10^6$ CFU/mL *T. interdigitale* after 7 days of incubation. Error bars represent the standard deviation from the mean of triplicate determinations. Different letters signify that the results are statistically significant ($p < 0.05$).

Figure 7.4: Log reduction cotton fabrics impregnated with Ag, Cu, Ag/Cu and Ag+Cu nanoparticles (a) synthesised in 50 mM metal salts and 3% (w/v) of chitosan (b) of same mean particle size of 200 nm impregnated into fabrics against $10^6$ CFU/mL *T. interdigitale* after 7 days of incubation. Error bars represent the standard deviation from the mean of triplicate determinations. Different letters signify that the results are statistically significant ($p < 0.05$).

Figure 7.5: FEGSEM images of the fungus *T. interdigitale* in contact with cotton fibre without nanoparticles at low magnification.

Figure 7.6: FEGSEM images of the fungus *T. interdigitale* bacterium in contact with cotton fibre (a) without nanoparticles (b) impregnated with Ag/Cu nanoparticles synthesised in 50 mM metal salts and 3 wt% of chitosan.
CHAPTER 1

INTRODUCTION

1.1 Background

Consumers strive to find the best quality products for their daily requirements. This includes clothing which is comfortable, and stays fresh and odour-free in use (Khan et al., 2011). Cotton fabrics are widely used in the production of underwear, protective clothing, medical garments, white goods and sportswear because of their breathability, moisture absorption and comfort (Filipowska et al., 2011; Kantouch & El-Sayed, 2008).

Microorganisms are part of our daily lives and environments. Bacteria are responsible for significant infections and allergy problems (SaIhi et al., 2005). Fabrics are frequently exposed to the influence of microorganisms, where the formation of spots and odours are the perceivable signs of contamination (El-Naggar et al., 2003). Microorganisms can also survive on fabric substrate (Hebeish et al., 2011). Fabrics made from natural fibres can act as carriers for microorganisms such as bacteria and mould (Lee, 2010). Natural fibres are more vulnerable than synthetics because of their porous, hydrophilic structures that can retain water, oxygen, and nutrients (Hebeish et al., 2011). Besides this, natural fibres in contact with the human body provide warmth which makes the best environment for microbial growth and multiplication (Prusty et al., 2010). These phenomena can lead to discolouration and deterioration of the quality of the fabric, unwanted stains, dermal infection, allergic reactions and other associated illnesses (Filipowska et al., 2011). The growing need for hygienic living environments has led to a great demand for antimicrobial materials which do not allow microbes to attach, survive or at least proliferate on material surfaces (Thomas et al., 2010).
Additionally, antimicrobial fabrics could have a major impact in hospitals due to growing concern over microbial cross-contamination in hospitals from the infected patients and indoor air quality in operational areas (Mohammadkhodaei et al., 2010). It has been claimed that, hygiene problems associated with hospital fabrics can affect the recovery of the patient (Zhao et al., 2008). It is believed that the use of antimicrobial fabrics can efficiently control and inhibit microbial contamination and the spread of disease (Copello et al., 2006). This would reduce the possibility of pathogenic bacteria being transported from the hands or gloves of a health care-worker to the wound site of an at-risk patient (Borkow & Gabbay, 2008). The occurrence of pathogenic bacteria in the hospital environment could be restricted by the utilisation of effective antimicrobial compounds on frequently touched surfaces, such as curtains (particularly around patient beds), uniforms and bedding (O’Hanlon & Enright, 2009).

The requirement for antimicrobial treatment is not limited to hospitals. Besides this, cross contamination by microorganisms can also happen in other places such as hotels, schools, nursing facilities, clinics and public areas (El.Shafei & Abou-Okeil, 2011). It mainly occurs in places where crowded conditions prevail and which do not have frequent cleaning (Hebeish et al., 2011). Therefore, the requirement for fabrics that are resistant to the growth of microorganisms and pathogens is extended (Yan et al., 2011).
1.2 Research problems

Microorganisms have been causing various types of diseases for thousands of years and people unable to avert them. An enhanced awareness and routine of infection control performs has had some influence on the existence of microorganism infections. The growth of microorganisms can be controlled by treatment with antimicrobial agents.

The requirement to use antimicrobial fabrics has been increasing all around the world (Lin et al., 2011). Antimicrobial fabrics are developed for three major reasons: (a) to prevent the spread of disease and avoid the danger of injury-induced infection, (b) to avoid the development of odour from perspiration, stains and soil on fabric materials, and (c) to prevent the deterioration of fabrics caused by mildew, particularly for fabrics made of natural fibres (Diana et al., 2010; Gao and Cranston, 2008).

Nowadays, industrial and textile sectors are paying increased attention to manufacturing antimicrobial fabrics for medical and hygienic applications (Thomas et al., 2010). As a result, different types of antimicrobial agents for fabric application have been created, but unfortunately many of these agents have harmful effects and cannot easily degrade in nature (Dastjerdi & Montazer, 2010). Producing new kinds of fabrics with antimicrobial properties using nanoparticles has attracted a great deal of attention from both scientists and consumers in recent years because of their non-toxic, safe and improved antimicrobial efficiency (Kim et al., 2010). Synthesis of metal nanoparticles has been studied extensively because of their antimicrobial activity (Marambio-Jones & Hoek, 2010).

Copper (Cu) is a potent natural antimicrobial material which has been used since ancient times. For example, Cu is used for storing potable water and along with vinegar and honey for cleansing wounds (Russell, 2002). Cu plays a vital role in human health, such as energy production in cells and the maintenance of essential elements and chemicals such as zinc, oestrogen, and neurotransmitters (Cady et al., 2014).
Chitosan can form electrostatic attraction with metal components thus enhancing the stability of the nanoparticles (Guibal, 2004; El.Shafei & Abou-Okeil 2011). It has low toxicity and it is therefore safe for human applications, and will not cause environmental-toxicity or biological hazards (Kong et al., 2010). Synthesis is inexpensive and leads to waste reduction and energy efficiency.

Modification of fabric with nanoparticles is developed due to their unique properties. However, the surface modification of fabric with nanoparticles is not permanent especially against washing. Most methods used for stabilization of nanoparticles on the fabrics are costly, very time-consuming and are harmful to the environment because of the application of hazardous chemicals or organic solvents (Dastjerdi & Montazer, 2010).

The development of antimicrobial fabrics based on synergistic effects through combining different metal elements provides a new alternative in the fight against various types of pathogenic microorganisms. This study will provide new insight into the antimicrobial activity of the nanoparticles through synergistic effects between Ag and Cu. The results can be used as a basis for further study of the application of the nanoparticle-impregnated fabrics to kill pathogenic bacteria and fungi. This study will contribute to the knowledge of the application of nanoparticle-impregnated fabrics to reduce undesirable phenomena caused by microorganisms.

1.3 Aim and objectives

The aim of this research work was to develop antimicrobial fabrics that have antibacterial and antifungal effects which involve the use of different types of nanoparticles impregnated onto cotton fabrics and a pre-treatment process. Various methods and analyses were used as reported in this thesis to achieve this aim, with the following objectives: