Antibacterial evaluation of activated carbon cloth with Ag\textsuperscript{+} impregnated with ZnO nanoparticles

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Abstract

Purpose – Staphylococcus aureus (S. aureus), Klebsiella pneumoniae (K. pneumoniae) and Streptococcus pneumoniae (S. pneumoniae) are among the pathogens detected during Hajj pilgrimage known to cause pneumonia. This study aims to evaluate the antibacterial activity of activated carbon cloth (ACC) with Ag\textsuperscript{+} impregnated with zinc oxide nanoparticles (ZnO NPs) against these pathogens.

Design/methodology/approach – ZnO NPs were impregnated into ACC-Ag\textsuperscript{+} via layer-by-layer (LbL) self-assembly. Scanning electron microscope (SEM) was used to observe the fine surface morphological details of the ACC-Ag\textsuperscript{+}-ZnO sheets. Antibacterial activity of the ACC-Ag\textsuperscript{+}-ZnO sheets was evaluated using the disk-diffusion susceptibility assay. Allergy patch test was done to evaluate allergic reactions of the ACC-Ag\textsuperscript{+}-ZnO sheets on human skin.

Findings – SEM micrographs showed successful impregnation of ZnO NPs into the ACC-Ag\textsuperscript{+} sheets. Disk-diffusion susceptibility assay results of ACC-Ag\textsuperscript{+}-ZnO sheets against S. aureus, K. pneumoniae and S. pneumoniae showed good antibacterial activity; with 1.82 ± 0.13 mm zone of inhibition for S. pneumoniae, at

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Conflict of interest: On behalf of all authors, the corresponding author states that there is no conflict of interest.
a ZnO concentration of 0.78 mg mL⁻¹. No signs of human skin irritation were observed throughout the allergy patch test.

**Originality/value** – Results indicate that ACC-Ag⁺-ZnO sheets could potentially be embedded within surgical face masks (pilgrims’ preferred) to reduce the risks involved with the transmission of respiratory tract infections during and after mass gatherings (e.g. Hajj/Umrah, Olympics).

**Keywords** Zinc oxide, Nanoparticles, Antibacterial, Activated carbon cloth, Allergy patch test, Disk-diffusion

**Paper type** Research paper

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**1. Introduction**

Every year, over two million Muslims of vast ethnic diversities congregate in Makkah-Saudi Arabia to perform Hajj (Assiri et al., 2018). The close contact among pilgrims in confined areas during Hajj increases the risks of disease transmission (Benkouiten et al., 2014a, 2014b). Therefore, the use of face masks is recommended by the Saudi health authorities (Saudi Ministry of Health, 2018). Surgical face masks reduce the transmission of infection by acting as a barrier for large respiratory droplets, but are not designed to seal tightly to users face and should not be relied on to reduce exposure to inhalable airborne particles. The N95 respirators have higher filtration efficiency and found to protect against bacterial and viral infections (3M Personal Safety Division, 2018). However, due to various reasons including a relatively higher degree of breathing discomfort when using the N95 respirators, majority pilgrims preferred the surgical face masks (Azman et al., 2017).

During the 2013 Hajj, about 82.9 per cent of 468 Malaysian pilgrims used one or other type of face masks during Hajj (Hashim et al., 2016). However, majority pilgrims (68.8 per cent) used surgical face masks while only a few (3.2 per cent) used the more efficient N95 respirators. This preferential use of surgical face masks was also seen with Malaysian Umrah pilgrims (Azman et al., 2017); 86.67 per cent used surgical face masks; and 6.67 per cent used N95 respirators. Nevertheless, infected face masks (either surgical or N95 type) could themselves become a source of infections if not disposed of properly. Nanoparticles provide larger surface areas thus, increasing the interactions with the microorganisms, and are being used to impart antimicrobial properties in face masks (Borkow et al., 2010; Li et al., 2006; Smith and Taylor, 2010).

The Pure Non-Scents® Ag⁺ face masks make use of microporous Zorflex® activated carbon cloth (ACC) impregnated with Ag⁺ for odor control and wide-ranging protection (Charcoal House LLC, 2018). Zorflex® ACC comprises long staple fibers that are twisted into a yarn, which, in turn, is knitted into a double jersey textile (Murphy, 2016). The microporous Zorflex® ACC can absorb a large volume of organic or inorganic molecules and is suitable for applications where air permeability is required (Chemviron Carbon Cloth Division, 2011). However, there is a lack of literature on the antimicrobial properties of these face masks against the pathogens commonly found during the Hajj. Also, it was found that the synergistic effect of two nanoparticle types impregnated into activated carbon produced improved antibacterial activity compared to a single nanoparticle type (Prashantha Kumar et al., 2015). So, it can be expected that the woven ACC with Ag⁺ impregnated with other metal or metal oxide nanoparticles (NPs) can provide improved antimicrobial activity.

Zinc oxide (ZnO) NPs are biocompatible, low-cost and non-toxic to the human cells at low concentrations (Jones et al., 2008) and are being used in wound dressing, food packaging, antimicrobial fabric, etc. (Asokan et al., 2010; Sudheesh Kumar et al., 2012). ZnO NPs effectively inhibit both the gram-positive and the gram-negative bacterial strains (Navale et al., 2015; Sirelkhatim et al., 2015; Yamamoto, 2001; Zhang et al., 2010). The woven ACC
with Ag\textsuperscript{+} impregnated with ZnO NPs can be embedded within the pilgrims’ preferred surgical face masks to impart antimicrobial characteristics in the masks while in use and prevent the spread of infection from improperly disposed of masks.

Therefore, this study aimed to evaluate the antibacterial activity of Zorflex\textsuperscript{®} woven ACC with Ag\textsuperscript{+} (ACC-Ag\textsuperscript{+}) impregnated with ZnO NPs using the simple electrostatic layer-by-layer (LbL) self-assembly method. The fine surface morphologies of the ACC-Ag\textsuperscript{+}-ZnO sheets were observed using scanning electron microscopy. The antibacterial activity of the ACC-Ag\textsuperscript{+}-ZnO sheets was evaluated via the disk-diffusion susceptibility test against some of the commonly found respiratory tract pathogens during Hajj, namely, \textit{Staphylococcus aureus} (\textit{S. aureus}), \textit{Klebsiella pneumoniae} (\textit{K. pneumoniae}) and \textit{Streptococcus pneumoniae} (\textit{S. pneumoniae})\textsuperscript{(Ahmed \textit{et al.}, 2006; AlBarrak \textit{et al.}, 2018; Al-Tawfiq \textit{et al.}, 2016; Benkouiten \textit{et al.}, 2014a, 2014b; Dzaraly \textit{et al.}, 2014; El-Sheikh \textit{et al.}, 1998; Mandourah \textit{et al.}, 2012; Memish \textit{et al.}, 2015; Shirah \textit{et al.}, 2017). The allergic reactions of ACC-Ag\textsuperscript{+}-ZnO sheets on human skin were evaluated using a semi-open allergy patch test. The ACC-Ag\textsuperscript{+}-ZnO sheets exhibited good antibacterial activity against \textit{S. aureus, K. pneumoniae} and \textit{S. pneumoniae}; while showing no signs of human skin irritation. It is anticipated that surgical face masks embedded with ACC-Ag\textsuperscript{+}-ZnO sheet could potentially reduce the risk of respiratory tract infections during Hajj/Umrah pilgrimage or other mass gatherings such as the Olympics.

2. Materials and methods
Zorflex\textsuperscript{®} ACC with 0.3 per cent Ag\textsuperscript{+} by weight was purchased from Charcoal House LLC (Nebraska, USA). The ACC-Ag\textsuperscript{+} sheets are made up of 100 per cent activated carbon and a woven cloth type (single weave fabric; one layer of fabric made with interlaced sets of horizontal and vertical yarns). The specifications of the ACC-Ag\textsuperscript{+} sheets are: thickness 0.5 mm, surface density 120 g m\textsuperscript{−2}, carbon tetrachloride activity 55-70 (per cent ww) and air permeability at 10 mm is 100 cm\textsuperscript{2} cm\textsuperscript{−2} s\textsuperscript{−1}. The ZnO NPs (99+ per cent, 10-30 nm) were purchased from US Research Nanomaterial Inc. (Houston, USA). Poly styrene sulfonate (PSS) (\textit{MW} \sim 70 kDa) and Mueller–Hinton Agar (MHA) were purchased from Sigma-Aldrich.

Figure 1 contains a flow chart of the experimental methods used in this study. A stock solution of ZnO NPs (200 mg mL\textsuperscript{−1}) was made by dissolving 20 g of ZnO NPs in 100 mL of sterile distilled water; including 4 g of PSS to enhance the stability of the ZnO NPs in the solution. The pH of the stock solution was adjusted to pH 11. The stock solution was vigorously stirred followed by sonication in an ultrasonicator (Labsonic M) for 30 min. All solutions were used immediately after their preparation.

The tested organisms were \textit{S. aureus} (ATCC 9144), \textit{K. pneumoniae} (ATCC 13883) and \textit{S. pneumoniae} (ATCC 6305). \textit{S. aureus} and \textit{K. pneumoniae} were sub-cultured on MHA and were grown in Mueller–Hinton broth, while \textit{S. pneumoniae} was sub-cultured on Columbia with 5 per cent horse blood agar and was grown in Todd-Hewitt broth. Microscopic examination and biochemical tests were used to re-identify the bacteria. The inoculum was prepared by picking \sim 3-5 isolated colonies, of same morphological type, from an overnight growth with a sterile cotton swab. The colonies were then transferred into 5 mL Mueller–Hinton broth in the case of \textit{S. aureus} and \textit{K. pneumoniae} and into Todd-Hewitt broth in the case of \textit{S. pneumoniae}. The turbidity of the inoculum was adjusted by adding sterile normal saline to achieve turbidity of 0.5 McFarland standards (\textit{1 \times 10\textsuperscript{8} CFU mL\textsuperscript{−1}}) (CLSI, 2012). The inoculum was subsequently incubated at 37°C for 24 h.

Impregnation of the positively charged ACC-Ag\textsuperscript{+} with the negatively charged ZnO NPs was done using the well-established electrostatic LbL self-assembly method (Fardad \textit{et al.}, 2007). Two-fold serial dilutions were made to obtain ZnO NPs solutions with concentrations...
of 100, 50, 25, 12.5, 6.25, 3.13, 1.56 and 0.78 mg mL$^{-1}$. ACC-Ag$^+$ sheets of size 3 cm × 3 cm were dipped into Petri dishes filled with different concentrations of ZnO NPs. The Petri dishes were sealed using parafilm and placed in a shaking water bath (Memmert GmbH & Co. KG) for 1 h at room temperature, with a horizontal shaking movement of 160 strokes per minute. The ACC-Ag$^+$-ZnO sheets were then dried in an oven (Memmert GmbH & Co. KG) at 95°C for 30 min. The dipping and drying steps were repeated for three times.

A JEOL JSM-6360LA scanning electron microscope (SEM) was used to confirm the impregnation of ACC-Ag$^+$ sheets with the ZnO NPs. The ACC-Ag$^+$-ZnO sheets were punched with a 6 mm puncher to obtain samples for SEM imaging. The punched sample sheets were attached to the sample holder stubs with double-sided adhesive carbon tape. An Auto Fine Coater was used to sputter coat gold onto all the samples before observing under the SEM.

The ACC-Ag$^+$-ZnO sheets were punched with a 6 mm puncher to obtain triplicate samples for the disk-diffusion susceptibility assay. To sterilize the ACC-Ag$^+$-ZnO sheets, they were sprayed with 70 per cent ethanol followed by a UV light exposure for 30 min. The MHA plates were inoculated with 100 μL of K. pneumoniae or S. aureus inoculum. Plates of Columbia agar with 5 per cent horse blood were inoculated with 100 μL of S. pneumoniae inoculum. The ACC-Ag$^+$ sheets impregnated with different concentrations of ZnO NPs were placed onto the inoculated plates. ACC-Ag$^+$ was used as a negative control (Pasquet et al., 2014). Gentamicin, Oxacillin and Penicillin-G disks were used as positive controls for K. pneumonia, S. aureus and S. pneumoniae, respectively (Prashantha Kumar et al., 2015). After incubation of the inoculated plates at 37°C for 24 h, the inhibition zone diameters were measured using a Mitutoyo digital caliper and the values were expressed in millimeter (mm).

Figure 2 was used as a reference table to interpret the level of antibacterial activity of the ACC-Ag$^+$-ZnO sheets [adapted from (Cheng et al., 2014; Pollini et al., 2009)].

A semi-open allergy patch test (ethics approval # UHREC/2016/4/008) was done to evaluate the allergic reactions on the human skin due to the ACC-Ag$^+$-ZnO sheets. A sample size of 38 was used for the allergy test (Li et al., 2006). For the patch test, the ACC-Ag$^+$-ZnO
sheets were cut to a size of 2 cm × 2 cm. To sterilize the ACC-Ag⁺-ZnO sheets, they were sprayed with 70 per cent ethanol and exposed to a UV light for 30 min. The samples were dipped into 0.9 per cent NaCl for 30 min before patched onto the skin of the volunteers. The ACC-Ag⁺-ZnO sheets (prepared using 100 mg mL⁻¹ ZnO NPs) were patched onto the backs or arms (based on preference) of the 38 volunteers by a dermatologist. The sample patches were covered with a hypoallergenic adhesive tape and marked using a marker. After 24 h, the sample patches were removed, and the dermatologist examined the marked skin area for the presence of any allergic reactions. At 72 h after sample patch removal, the volunteers were re-examined by the dermatologist.

All the data are expressed as the mean ± standard error. An online unpaired *t*-test calculator (www.graphpad.com/quickcalcs/ttest1.cfm) was used to compute the *p*-value and the statistical significance. A 95 per cent confidence interval for the difference in population means was assumed.

3. Results and discussion

The ZnO NPs stock solution (200 mg mL⁻¹) appeared white and opaque to the naked eye after 30 min of ultrasonication. Figure 3 contains SEM micrographs of a pristine ACC-Ag⁺ sheet and ACC-Ag⁺-ZnO sheets at 30 × magnification. It is clear from these low magnification SEM micrographs that there is a higher degree of impregnation with increasing ZnO NP concentrations.

The SEM micrograph of ACC-Ag⁺-ZnO sheets impregnated at a concentration of 100 mg mL⁻¹ (Figure 3b) shows that most of the ACC inter-fiber gaps are getting filled-up with the ZnO NPs. Figure 4 contains SEM micrographs of a pristine ACC-Ag⁺ sheet and ACC-Ag⁺-ZnO sheets at 2,000 × magnification. As observed in Figure 3, these high magnification SEM micrographs show that a higher degree of impregnation is achieved at higher ZnO NP concentrations. The individual ACC fibers are not visible in the SEM micrograph in Figure 4b, showing the ACC-Ag⁺ sheets impregnated with ZnO NPs at a concentration of 100 mg mL⁻¹. At a concentration of 25 mg mL⁻¹, it appears from the SEM micrograph in Figure 4d that the individual ACC fibers are entirely being covered with ZnO NPs. The SEM micrograph in Figure 4i shows a sparse surface coverage of individual ACC fibers with ZnO NPs at a concentration of 0.78 mg mL⁻¹. The SEM micrographs in Figure 3 and Figure 4 demonstrate that the positively charged ACC-Ag⁺ sheets were successfully impregnated with the negatively charged ZnO NPs using the LbL self-assembly method. However, further characterization (e.g. FESEM, TEM and XRD) of the ACC-Ag⁺-ZnO sheets is required for visualization of the samples at higher magnifications and to determine the actual concentration of ZnO NPs impregnated into the ACC-Ag⁺-ZnO sheets. Furthermore,
Figure 3.
SEM micrographs of a pristine ACC-Ag sheet and ACC-Ag sheets impregnated with ZnO NPs at (a) concentration of (b) 100 mg mL$^{-1}$, (c) 50 mg mL$^{-1}$, (d) 25 mg mL$^{-1}$, (e) 12.5 mg mL$^{-1}$, (f) 6.25 mg mL$^{-1}$, (g) 3.13 mg mL$^{-1}$, (h) 1.56 mg mL$^{-1}$ and (i) 0.78 mg mL$^{-1}$ (at 30 × magnification)
Figure 4.
SEM micrographs of a pristine ACC-Ag sheet and ACC-Ag sheets impregnated with ZnO NPs at (a) concentration of (b) 100 mg mL$^{-1}$, (c) 50 mg mL$^{-1}$, (d) 25 mg mL$^{-1}$, (e) 12.5 mg mL$^{-1}$, (f) 6.25 mg mL$^{-1}$, (g) 3.13 mg mL$^{-1}$, (h) 1.56 mg mL$^{-1}$ and (i) 0.78 mg mL$^{-1}$ (at 2,000 $\times$ magnification)
such additional characterization techniques would be useful in studying the reproducibility and lifetime of the ACC-Ag$^+$-ZnO sheets.

The ACC-Ag$^+$-ZnO sheets showed antibacterial properties against the three tested bacteria as evidenced by the disk-diffusion method. The disk-diffusion susceptibility assay results of the ACC-Ag$^+$-ZnO sheets against *S. aureus*, *K. pneumoniae* and *S. pneumoniae* are summarized in Table I. The inhibition zone diameters (in millimeters) have been expressed as the mean ± standard error ($n = 3$). The zone of inhibitions is presented as the diameter of the clear area with no bacterial growth, less the diameter of the sample (Cheng et al., 2014). However, for the antibiotics, the zone of inhibitions is presented as the diameter of the clear area with no bacterial growth. The level of antibacterial activity was interpreted to be “susceptible”; “intermediate”; or “resistant” based on earlier reports for Oxacillin (Loomba et al., 2010), Gentamicin (Manikandan and Amsath, 2013) and Penicillin G (Jacobs et al., 1979). It is clear from Table I that there is an increasing zone of inhibition with increasing ZnO NP concentrations.

From the data in Table I, it is clear that the ACC-Ag$^+$-ZnO sheets displayed the highest antibacterial activity against *S. pneumoniae*. The ACC-Ag$^+$ sheets impregnated with ZnO NPs at a concentration of 0.78 mg mL$^{-1}$ started to show the signs of inhibition, indicated by the clear inhibition zone with a diameter of 1.82 ± 0.13 mm. Also, ACC-Ag$^+$-ZnO sheets showed good inhibition activity against *S. aureus*. However, the signs of inhibition only started with ACC-Ag$^+$ sheets impregnated with ZnO NPs at a concentration of 12.5 mg mL$^{-1}$.

<table>
<thead>
<tr>
<th>ZnO NPs concentration (mg mL$^{-1}$) or controls</th>
<th>Zone of inhibition (mm)(mean ± standard error)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>S. aureus</em></td>
</tr>
<tr>
<td>100</td>
<td>$2.72 ± 0.50^{**}$</td>
</tr>
<tr>
<td></td>
<td>Good</td>
</tr>
<tr>
<td>50</td>
<td>$1.40 ± 0.70^{*}$</td>
</tr>
<tr>
<td></td>
<td>Good</td>
</tr>
<tr>
<td>25</td>
<td>$1.13 ± 0.58$</td>
</tr>
<tr>
<td></td>
<td>Good</td>
</tr>
<tr>
<td>12.5</td>
<td>$0.98 ± 0.49$</td>
</tr>
<tr>
<td></td>
<td>Fairly good</td>
</tr>
<tr>
<td>6.26</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Sufficient</td>
</tr>
<tr>
<td>3.13</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Sufficient</td>
</tr>
<tr>
<td>1.56</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Sufficient</td>
</tr>
<tr>
<td>0.78</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Sufficient</td>
</tr>
<tr>
<td>ACC-Ag$^+$</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Sufficient</td>
</tr>
<tr>
<td>Oxacillin</td>
<td>$31.47 ± 0.60$</td>
</tr>
<tr>
<td></td>
<td>Susceptible</td>
</tr>
</tbody>
</table>

**Notes:** *Statistically significant; **very statistically significant (95% CI)*

Table I. Summary of the disk-diffusion assay results of the ACC-Ag$^+$ sheets impregnated with ZnO NPs against *S. aureus*, *K. pneumoniae* and *S. pneumoniae*.
mL⁻¹; with inhibition zone with a diameter of 0.98 ± 0.49 mm. The weakest antibacterial activity for the ACC-Ag⁺-ZnO sheets was seen against the Gram-negative bacteria, *K. pneumoniae*. In the case of *K. pneumoniae*, no zones of inhibition were observed for the ACC-Ag⁺-ZnO sheets impregnated with all tested concentrations of ZnO NPs. Nevertheless, it was found that no bacterial growth occurred on the sample surfaces.

In the disk-diffusion assay with blank disks infused ZnO NPs solutions, it was found that the gram-positive bacterial strains are more susceptible to ZnO NPs compared to the gram-negative bacterial strain; with *S. pneumoniae* found to be the most vulnerable. A similar observation can be made from the data in Table I. The ACC-Ag⁺-ZnO sheets are more effective against the gram-positive bacterial strains than against the gram-negative bacterial strain. These observations are consistent with previous studies (Azam *et al.*, 2012; Premanathan *et al.*, 2011), where it was found that the effect of ZnO NPs was more prominent against the gram-positive bacterial strains compared to the gram-negative bacterial strains.

During the semi-open allergy patch test, the volunteers were examined for signs of skin allergy after wearing the ACC-Ag⁺-ZnO sheets for 24 h. All the 38 volunteers showed no signs of local skin inflammation and did not complain of any itchiness. Similar observations were made during the re-examination conducted at 72 h after sample patch removal. Figure 5 contains a representative photograph from the allergy test taken 72 h after the ACC-Ag⁺-ZnO patch was removed. The representative picture shows no sign of skin inflammation or irritations. An earlier study by (Li *et al.*, 2006) also reported similar observations; where nanoparticles (a mixture of silver nitrate and titanium dioxide) coated into face mask did not cause local skin allergy or inflammation in volunteers.

Overall, the results show the possibility of embedding the antibacterial ACC-Ag⁺-ZnO sheet within the layers of the pilgrims’ preferred surgical face masks. The additional air permeable (Chemviron Carbon Cloth Division, 2011) layer could impart antibacterial characteristics in the surgical face masks while in use and could prevent improperly disposed of face masks from
becoming the secondary source of transmission. The antibacterial surgical face masks could potentially reduce the risk of respiratory tract infections among pilgrims during the pilgrimage and prevent the transmission of infections to non-pilgrims when the pilgrims return to their respective countries. The improved face masks may also be useful to other mass gatherings such as the Olympics or other closed settings such as hospitals, schools, airports, airplanes and ships. The antibacterial ACC-Ag\textsuperscript{+}-ZnO sheets could also be used in other applications, e.g. sanitary products.

4. Conclusion
In conclusion, the LbL self-assembly technique was successfully used to impregnate ZnO NPs into the ACC-Ag\textsuperscript{+} sheets. At the different ZnO NP concentrations tested in this study, the ACC-Ag\textsuperscript{+}-ZnO sheets displayed antibacterial activity against \textit{S. aureus}, \textit{K. pneumoniae} and \textit{S. pneumoniae}. The ACC-Ag\textsuperscript{+}-ZnO sheets were most effective against the gram-positive bacterial strains. At the lowest concentration (0.78 mg mL\textsuperscript{-1}) tested, the ACC-Ag\textsuperscript{+}-ZnO sheets displayed the highest antibacterial activity against \textit{S. pneumoniae}. The ACC-Ag\textsuperscript{+}-ZnO sheets did not elicit any skin inflammation or irritation. The results of the current study are in agreement with previous reports that demonstrated the antimicrobial properties of activated carbons impregnated with metal or metal oxide NPs. Based on the preliminary results, it can be deduced that the ACC-Ag\textsuperscript{+}-ZnO sheet has potential use as a layer in the surgical face masks, preferred by most Hajj pilgrims. It is anticipated that face masks containing the ACC-Ag\textsuperscript{+}-ZnO sheet would benefit in minimizing the risk of respiratory tract infections during Hajj/Umrah pilgrimage or other mass gatherings such as the Olympics.

References


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