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# Highly Porous Chitosan Beads Embedded with Silver-Graphene Oxide Nanocomposites for Antibacterial Application

(Manik Kitosan Berliang Tinggi yang Dibenamkan dengan Nanokomposit Argentum-Grafin Oksida untuk Aplikasi Antibakteria)

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ABSTRACT

*Incorporation of silver nanomaterial into polymer matrix can further accomplished their potential usage in real life applications. In our previous study, silver nanoparticles (AgNPs) and silver-graphene oxide nanocomposites (AgGO) were prepared via a rapid microwave-assisted method. Hereby, the as-synthesized AgNPs or AgGO was dispersed in a chitosan solution. Subsequently, the resultant mixture solution was further coagulated in a coagulation bath containing sodium hydroxide via a neutralization process. This resulted in the formation of spherical-shaped chitosan beads. The structure of the beads showed that the chitosan beads embedded with AgGO exhibited a more porous structure as compared to the plain chitosan beads. Furthermore, the chitosan beads containing AgNPs or AgGO were tested for their antibacterial activity against* Escherichia coli *and* Staphylococcus aureus*. The antibacterial results indicated that the silver nanomaterial contained chitosan beads could effectively inhibit the growth of both* E. coli *and* S. aureus *as compared to the bare chitosan beads. The produced chitosan nanocomposite envisioned that can be potentially employed for water disinfection purpose.*

*Keywords: Antibacterial; chitosan; silver-graphene oxide nanocomposites; silver nanoparticles*

ABSTRAK

*Penggabungan nanobahan Argentum ke dalam matrik polimer boleh dicapai bagi potensi kegunaan dalam aplikasi kehidupan sebenar. Dalam kajian kami sebelum ini, nanozarah Argentum (NZAg) dan nanokomposit Argentum-grafin oksida (AgGO) telah disediakan melalui kaedah berbantu mikrogelombang pantas. Dengan ini, NZAg atau AgGO yang terhasil telah disebar ke dalam larutan kitosan. Kemudiannya, hasil larutan campuran seterusnya digumpal dalam rendaman penggumpal yang mengandungi natrium hidroksida melalui proses peneutralan. Ini menyebabkan pembentukan manik kitosan berbentuk sfera. Struktur manik menunjukkan manik kitosan yang dibenamkan dengan AgGO mempamerkan struktur yang lebih berliang berbanding manik kitosan biasa. Tambahan lagi, manik kitosan yang mengandungi NZAg dan AgGO telah diuji untuk aktiviti antibakteria terhadap* Escherichia coli *dan* Staphylococcus aureus*. Keputusan antibakteria menunjukkan bahawa manik kitosan mengandungi nanobahan Argentum berkesan menghalang pertumbuhan kedua-dua* E. coli *dan* S. aureus *berbanding manik kitosan biasa. Nanokomposit kitosan yang terhasil diharapkan dapat berpotensi untuk digunakan bagi tujuan pembasmian kuman air.*

*Kata kunci: Antibakteria; kitosan; nanokomposit Argentum-grafin oksida; nanobahan Argentum*

## Introduction

For the past two decades, there is an increasing number of research regarding silver nanoparticles (AgNPs) due to their excellent antibacterial activity as compared to bulk silver particles. The high surface area to volume ratio of AgNPs enhanced contact rates with the bacterial cell wall (Panáček et al. 2006; Rai et al. 2009). The release of $Ag^+$ ions has been recognized as one of the main mechanisms for the antibacterial activity of AgNPs, causing cell membrane lysis and formation of reactive oxygen species (ROS), which would led to damage on replication system (such as DNA) of the bacteria (Chaloupka et al. 2010). The emerging interest in graphene-based nanocomposites incorporated with metallic nanoparticles due to their interesting properties for electrical, biosensor and biomedical applications (Shen et al. 2010; Zainy et al. 2012). Great number of studies demonstrated that AgGO nanocomposite exhibited a comparable or better antibacterial performance than bare AgNPs due to the synergistic effect between AgNPs and GO (Chook et al. 2012; Liu et al. 2011; Ma et al. 2011).

Chitosan, a nontoxic, biocompatible, abundant and biodegradable polymer found in animals and crustaceans, is derived from the deacetylation of chitin (Jayakumar et al. 2010; Ravi Kumar 2000). Chitosan has been widely studied for potential use in wound dressing and food packaging materials due to its antibacterial properties (Bordenave et al. 2009; Jayakumar et al. 2011). Study has shown that chitosan were able to inhibit the growth of Gram positive bacteria such as *S. aureus* effectively (No et al. 2002). Many researchers have reported the antibacterial properties of composite consisting chitosan and AgNPs (Ali et al. 2011; López-Carballo et al. 2012; Sanpui et al. 2008; Vimala et al. 2010). Hereby, chitosan beads embedded with AgNPs or AgGO were produced through a simple solution mixing and coagulating process. The synergistic antibacterial effects of both chitosan and Ag nanomaterials towards *Staphylococcus aureus* and *Escherichia coli* bacteria were investigated.

## Materials and Methods

Analytical grade silver nitrate ($AgNO_3$), sodium hydroxide (NaOH), ammonium hydroxide ($NH_4OH$, 25%) and glucose used in this study were purchased from Merck. Medium molecular weight chitosan with 75-85% degree of deacetylation was purchased from Sigma Aldrich. GO was prepared using the simplified Hummer's method (Chook et al. 2012).

### Preparation of Chitosan Beads

A chitosan solution 2.0% (w/v) in 2.5 wt. % acetic acid solution was prepared. The chitosan solution was slowly dropped into a coagulation bath containing 1.0 M NaOH solution using a pipette to form spherical shaped chitosan bead (CB). The beads were collected after 4 h of coagulation and then washed repeatedly with deionized water to remove excessive acetic acid and NaOH. AgNPs and AgGO were prepared using a microwave irradiation-assisted method as described previously (Chook et al. 2012). In order to form chitosan beads embedded with GO, AgNPs or AgGO, each nanomaterial was separately well-dispersed into chitosan solution to obtain a resulting solution with a concentration of 100 μg/mL. The prepared AgGO nanocomposites has 40 wt. % of Ag (Chook et al. 2012), hence the actual total Ag content for CB-AgGO is approximately 40 μg/mL. Each mixture was coagulated in 2 M NaOH solution to produce chitosan beads containing GO (CB-GO), AgNPs (CB-AgNPs) and AgGO (CB-AgGO). The beads were washed and freeze-dried for 24 h. The physical structure of the beads was observed using Field emission scanning electron microscopy (FESEM, Zeiss Supra 55VP).

### Antibacterial Test

Chitosan beads containing GO, AgNPs and AgGO were tested for antibacterial activity against Gram-positive (*S. aureus*) and Gram-negative bacteria (*E. coli*). An amount of 20 beads was added into tubes containing nutrient broth that have been inoculated with 105 CFU of bacteria, followed by incubation in an incubator shaker at 37°C. A control sample was prepared by incubating nutrient broth inoculated with the same CFU of bacteria under the same conditions without adding any bead samples. After 4 h of incubation, the turbidity of the broth was visually inspected for bacterial growth. In the meantime, 0.1 mL of the broth was withdrawn and spread on a nutrient agar plate and incubated at 37°C for 24 h.

## Results and Discussion

Briefly, the chitosan beads were formed as the acidic chitosan solution was neutralized with the NaOH solution. The diffusion of the NaOH solution into the chitosan and its mixture containing nanomaterial has induced the regeneration and crosslinking process of the chitosan structure (Araiza et al. 2008). The FESEM images of the produced beads structure are shown in Figure 1. Both CB (Figure 1(a)) and CB-AgNPs (Figure 1(b)) possessed rigid and closed structure, while the CB-GO (Figure 1(c)) and CB-AgGO (Figure 1(d)) beads exhibited a highly porous structure. This could be due to the addition and interaction of GO with chitosan has created a more porous structure of chitosan network during the coagulation process.

Generally, both CB-AgNPs and CB-AgGO demonstrated strong antibacterial activity against *S. aureus* and *E. coli* after 4 h of incubation. Prior to the test, nutrient broth inoculated with *S. aureus* or *E. coli* appeared to be clear and transparent, as shown in Figure 2(a) and 2(c). The growth of bacteria can be visually observed by the changes in the turbidity or cloudiness of the broth. Turbid broth signifies that the bacteria have grown rigorously, while clear broth indicates non-growing result. After 4 h of incubation, the broth treated with CB and CB-GO beads were more turbid, suggesting that the CB and CB-GO did not exhibit antibacterial effect. As compared to CB-AgNPs and CB-AgGO beads which showed a stronger antibacterial activity, the treated broth remained clearer, as shown in Figure 2(b) and 2(d).

Furthermore, the bacteria colony count of the spread plate results is summarized in Table 1, which is consistent with the turbidity observation. Despite chitosan has been widely reported for its antibacterial property, pure chitosan beads showed negligible antibacterial effect against both Gram-positive and Gram-negative bacteria relative to control sample. Additionally, CB-GO also shows negative inhibition effects against both bacteria, which is consistent with our previous study (Ali et al. 2011). On the contrary, the spread plating results for CB-AgNPs and CB-AgGO showed a much significant growth inhibition for both bacteria tested, where lesser bacterial colonies were observed and counted on the plates as compared to the control sample. Both visual inspection of broth turbidity and the spread plating results suggested that the AgNPs- and AgGO-embedded chitosan beads effectively suppressed the growth of *S. aureus* and *E. coli* within 4 h of treatment. Interestingly, chitosan beads embedded with both Ag nanomaterials exhibited stronger antibacterial activity against *S. aureus* compared to our previous study (Chook et al. 2012). The results suggested that this is attributed to a synergistic effect between chitosan and Ag nanomaterials on *S. aureus* (Potara et al. 2011).

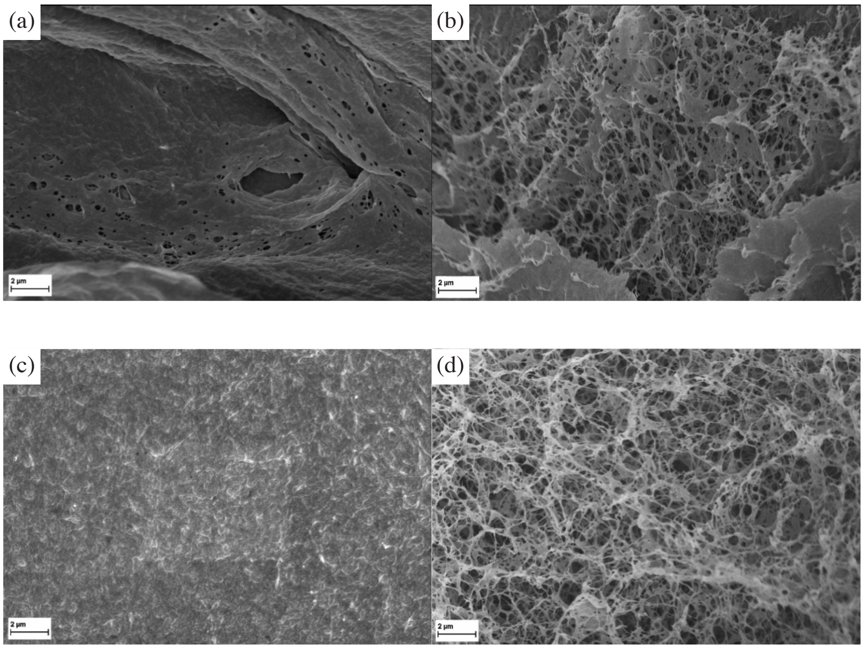


FIGURE 1. FESEM images of (a) CB, (b) CB-GO, (c) CB-AgNP and (d) CB-AgGO

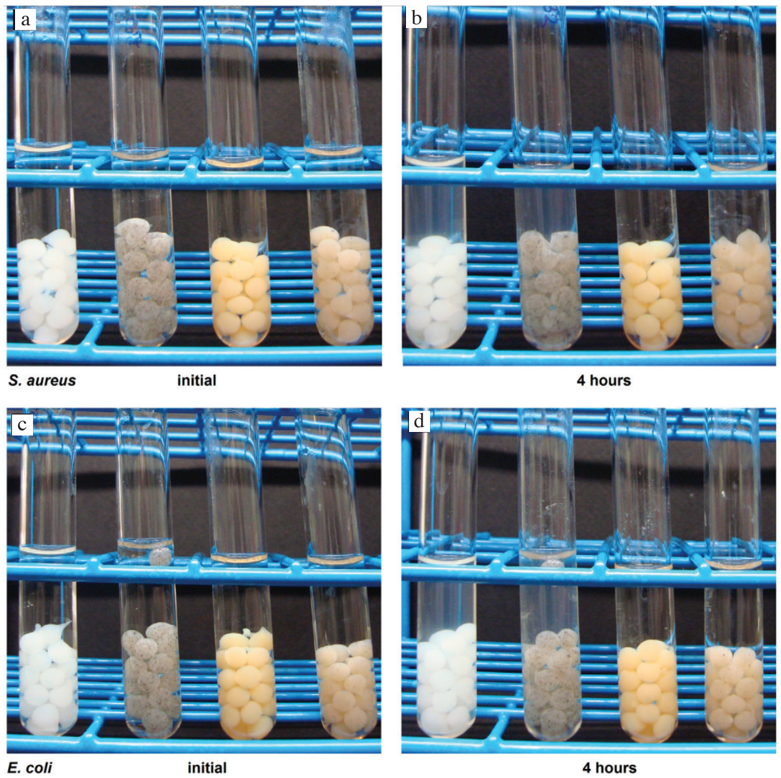


FIGURE 2. Visual inspection for turbidity of nutrient broth solution inoculated with *S. aureus* (a, b) and *E. coli* (c, d) treated with different chitosan beads. Tubes from left to right: CB; CB-GO; CB-AgNP and CB-AgGO

Due to the incorporation of AgNPs and AgGO embedded within the chitosan beads which limited the direct interaction of the nanomaterials with bacterial, the antibacterial activity of both chitosan beads postulated based on the dissolution and release of $Ag^+$ ions to inhibit the bacterial growth. Although with a lower Ag content as compared to CB-AgNPs, the CB-AgGO exhibited comparable antibacterial activity against both Gram-positive and Gram-negative bacteria. This is attributed to the more porous of the AgGO-embedded chitosan beads (as shown in Figure 1(d)), leading to more interactions between the bacteria and beads. In addition, $Ag^+$ ions were

TABLE 1. Bacterial colonies count after 4 h exposure of the bacterial cells to chitosan beads (CB) and chitosan-nanomaterial beads (CB-GO, CB-AgNP and CB-AgGO)

| Bacteria | Chitosan bead samples (CFU/mL) | | | | |
|---|---|---|---|---|---|
| | Control | CB | CB-GO | CB-AgNP | CB-AgGO |
| *S. aureus* | BL | BL | BL | 0 | 0 |
| *E. coli* | BL | BL | BL | $2 \times 10^6$ | $13 \times 10^6$ |

BL indicates formation of bacteria lawn

readily released upon the oxidation of the AgNPs once the exposure to water or air (Xiu et al. 2012) via the porous structure of the beads.

## CONCLUSION

Both CB-AgNPs and CB-AgGO beads showed effective antibacterial activity against both *S. aureus* and *E. coli* within a short period of time. In this study, AgNPs served as a reservoir for the release of Ag ions and diffuse through the chitosan matrices. Despite a lower content of Ag, CB-AgGO exhibits bacterial inhibition effect which comparable to CB-AgNPs. The presence of GO has resulting to a highly porous structure of the CB-AgGO beads, which facilitated more interactions between bacterial cells and Ag ions released from the embedded AgNPs.

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