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The antimicrobial effect of open-cell silver foams

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Abstract Open-cell silver foams with various pore sizes (494, 337, 126 and 39 μm) and porosity (60, 70 and 80 vol%) were produced using a powder-based replication method. It was found that the foams have strong microbial reduction efficiency. The antimicrobial effect of the foams is much stronger on gram-negative bacterium (*Escherichia coli*) than gram-positive bacterium (*Staphylococcus aureus*). With equivalent volume addition of NaCl particles, higher antimicrobial effect was found for Ag foams with larger pore size. The difference on antimicrobial effect between silver foams with various pore sizes is smaller when porosity of the foams increases from 60 to 80%. No correlation between particle sizes of NaCl and bacterial growth inhibition was found. In addition, effect of particle sizes and pore sizes of the foams on the bacterial growth inhibition is not as much as the effect of particle addition. It is expected that the positively charged Ag ions released from the surface of Ag foam structure would alter the morphology of bacteria strains in which disruption of cell wall and eventually damage were implemented.

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1 Introduction

Open-cell metallic foams can be utilised in many functional applications that benefit from the presence of interpenetrating pores and high internal surface area of foam materials, such as filter, heat exchangers, bone-replacement implants, catalyst supports and sound insulators [1–5]. Fabrication of open-cell metallic foams can be made by many methods, but the replication process through powder metallurgy route is preferable due to the ability to control the pore architecture by careful selection of space-holders [6]. Zhao and Sun [5, 6] developed a promising technique known as the sintering and dissolution process (SDP) at which NaCl is used as space holders. However, using NaCl may lead to corrosion of metal matrix if residual NaCl exists in cellular structure, resulting in a decrease in mechanical properties. Another technique recently developed by Jiang et al. [7, 8] has been reported on the use of carbamide as space holders to obtain more uniform foam structure and better mechanical properties. Other metals can also be used to produce open-cell foams. A recent development of silver (Ag) foams from our previous study shows that the foams have several promising physical and mechanical properties [7]. Based on these techniques, pore morphology and pore sizes in foams can be controlled by selecting a suitable space holder which can be easily removed either before or after sintering.

It is known that Ag is one of antimicrobial metals which is widely used as a bactericidal agent for medical applications such as for wounds and burns [8–13]. Recently, several researches show that silver nanoparticle is a very promising material for antimicrobial applications [14–17]. Ag can disrupt critical functions in a pathogen in which it cannot mutate to avoid this effect. It is generally believed that the interaction of Ag ion with thiol (–SH) groups

results in the bacteria inactivation [11]. The binding reaction of Ag can change the molecular structure of the macromolecule, rendering it worthless to the microbial cells. Inactivation of critical physiological functions, such as cell-wall synthesis, membrane transport, nucleic acid (such as RNA and DNA) synthesis and translation, protein folding and function, and electron transport, which is important in generating energy for the cell, is majorly affected when the cells are in contact with Ag [18]. Owing to the dysfunction of cell, the bacteria is either inhibited from growth or killed [9]. In addition, Ag is extremely active in small quantities. For certain bacteria, as little as one part per billion of silver may be effective in preventing cell growth [19].

The present study aims to investigate the antibacteria action of Ag foams against *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*). The difference in foam structure in relation with antimicrobial effect is also examined.

2 Experimental procedure

Open-cell Ag foams were produced using a powder-based replication method in which NaCl particle was employed as space holder. Ag powder was prepared by the reduction of silver nitrate through glycerol process. The complete description of Ag powder fabrication process can be found in the previous study [3]. To produce Ag foams, the Ag powder was homogeneously mixed with 60, 70 and 80 vol% NaCl particles of various sizes, in a total weight of 1.5 g, followed by a uni-axial cold compaction to a pressure of approximately 200 MPa in a lubricated 11-mm cylindrical tool steel die. The green compacts were placed in an alumina crucible and sintered in a pre-heated furnace at 750°C for 3 h, in air, in order to strengthen Ag-matrix network. After sintering, the samples were cooled down in air to room temperature. The NaCl particles were removed by dissolution in hot water at 95°C for 5 h. The relative foam density was calculated by measuring a foam diameter, height and weight, and compared with the density of pure Ag. Foam specimens were half-sectioned in vertical direction using electro discharge machining (EDM).

Ag foam specimens were sterilized using autoclave at 121°C for 15 min. *S. aureus* (ATCC6538) and *E. coli* (ATCC25922) were used as a model for gram-positive and gram-negative bacterias, respectively. Bacteria cultures were incubated at 37°C for 24 h in nutrient broth (NB). Bacterial dilution was made at 10^8, 10^7, 10^6 and 10^5 CFU ml^{-1}, followed by the addition of the foam specimens to NB at 10^5 CFU ml^{-1}. Activated samples were mixed using a shaking incubator at 37°C for 15 min. Serial dilutions of microbial were made at 10^1 and 10^2 CFU ml^{-1} and incubated at 37°C for 24 h. Amounts of viable bacteria were calculated as the number of bacteria per specimen as follows.

$$\% \text{ Reduction} = \frac{(A - B) \times 100}{A} \quad (1)$$

where A = number of viable bacteria in control medium (CFU ml^{-1}), B = number of viable bacteria in sample (CFU ml^{-1}). In the antimicrobial study, three foam specimens were tested for each experimental condition.

Microstructural examination of foam structure was performed using a JSM-6400 scanning electron microscope (SEM). Microbial morphology after incubation with Ag foams was observed by another SEM (JEOL, JSM-5410LV). Each microbe, approximately 10^8 CFU ml^{-1}, was mixed with Ag foams. Bacterial suspensions before exposure with Ag foams were used as a negative control. The specimen was prepared for SEM as followed. The bacterial suspensions were gently passed through a syringe to settle on the filter. After a brief rinse in the proper solution, glutaraldehyde was used to fix followed by osmium tetroxide. The cells were left in contact with the monolayer for 15 to 60 min. After rinsing with distilled water, the cell was then dehydrated by using ethanol. The samples were dried by critical point drying with CO_2. The

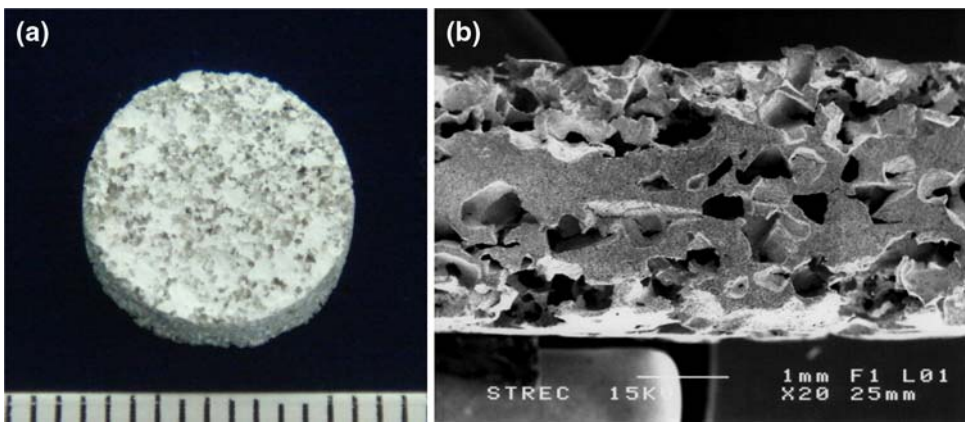

Fig. 1 **a** Ag foam specimen made using 70 vol% NaCl (494 μm) and **b** SEM micrograph showing the Ag foam microstructure

filter was glued on stages and coated with gold. The samples were then imaged with SEM. Statistical analysis was performed based on the data presented in mean values ± standard deviation (SD). Significance of difference was evaluated using Student's *t*-test and one-way ANOVA at the probability level of 0.05.

3 Results and discussion

Figure 1 presents an example of Ag foam made using 337 μm NaCl particles of 70 vol% and its cross-sectional cellular structure. It is clear that the foam has homogeneous structure with large interconnected porosity. The pore morphology and pore size of the foam are replicated from the morphology and size of NaCl particle. The pore distribution is rather uniform. No visible residual NaCl was observed on the surface of foam cross section.

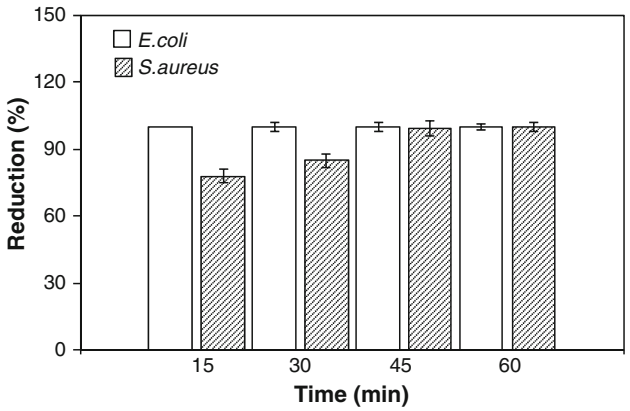

Fig. 2 Growth inhibition plots of *E. coli* and *S. aureus* in NB medium after exposing to Ag foam made using 494 μm NaCl particles of 70 vol% at varying exposure times

Figure 2 presents the effectiveness of bacterial reduction of Ag foam made using 70 vol% NaCl (494 μm) at varying exposing times. It was found that the Ag foam had the strongest inhibition effect on *E. coli* for all exposing times. However, the growth inhibition of *S. aureus* increased when the time increased and the inhibition was steady after 45 min. This is due to a presence of positively charged Ag ions on the surface of Ag foam with increasing time, resulting in further disruption of bacteria functions and growth inhibition. The disruption process of Ag foam is more effective to *E. coli* because the molecular structure of gram-negative bacteria is typically less complicated than that of gram-positive bacteria [18]. The membranes of *E. coli* contain negatively charged phosphate groups, which can attract more of positively charged Ag ions, resulting in phosphate uptake and exchange efflux of accumulated phosphate. The cell membrane of gram-negative bacteria as *E. coli*, is also generally thinner. According to these microorganism characteristics, critical functions in the bacterial cell, such as cell-membrane synthesis, membrane transport, nucleic acid synthesis and translation, protein folding and function, and electron transport, are better inactivated by Ag ions. As a result, *E. coli* strain is much easier to be growth inhibited or killed.

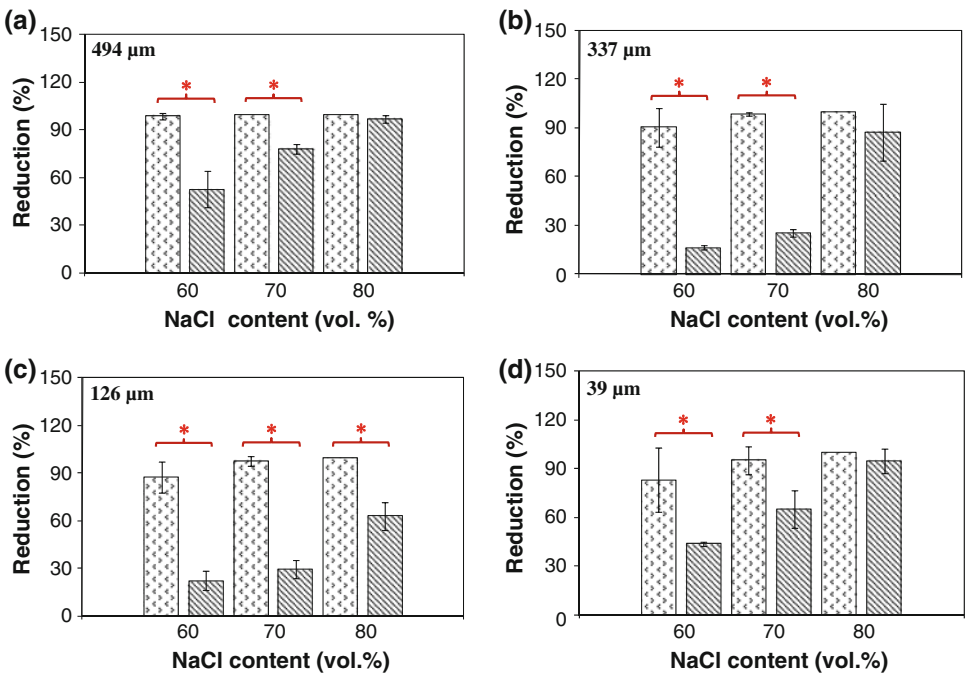

Fig. 3 Growth inhibition plots of *E. coli* and *S. aureus* in NB medium after exposing to Ag foams made using 60, 70 and 80 vol% NaCl of: **a** 494 μm, **b** 337 μm, **c** 126 μm and **d** 39 μm for 15 min (▨ *E. coli* and ▧ *S. aureus*). * The mean difference is significant at the 0.05 level

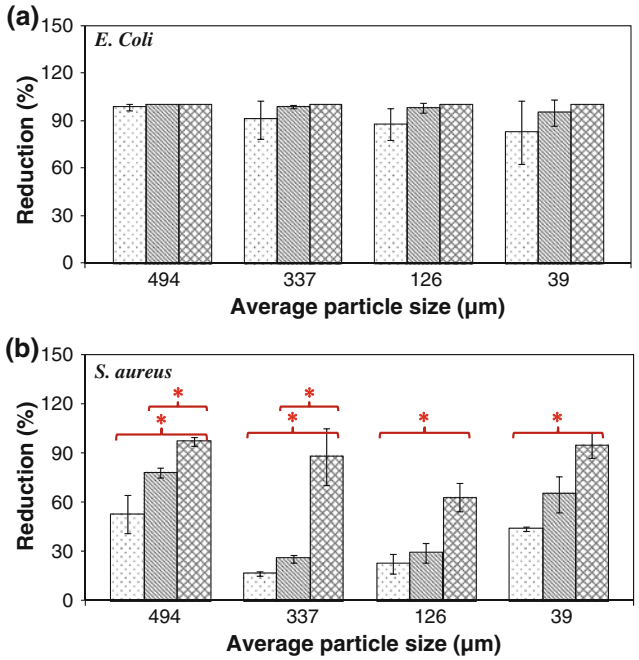

Fig. 4 Growth inhibition plots of **a** *E. coli* and **b** *S. aureus* in NB medium after exposing to Ag foams made using NaCl particles of 60, 70 and 80 vol% for 15 min (□ Ag + 60 vol% NaCl foam, ▨ Ag + 70 vol% NaCl foam and ▩ Ag + 80 vol% NaCl foam). * The mean difference is significant at the 0.05 level

Figure 3 demonstrates the growth inhibition of *E. coli* and *S. aureus* exposed to Ag foams made using 494, 337, 126 and 39 μm NaCl of various particle contents. In all cases, the growth of *E. coli* were effectively inhibited better than *S. aureus* (p value < 0.05), except for 80 vol% NaCl content. At 80 vol% NaCl addition, there is no significant difference on the inhibition efficiency (p value > 0.05). An increase in the volume fraction of NaCl particles, then porosity of the foam, resulted in an increase in the growth inhibition for both bacterias. This effect can be explained by the fact that with an increasing amount of porosity a higher binding reaction area between positively charged Ag ions and negatively charged side groups on bacterias through larger surface area of the porous foam structure is obtained. There is also a better access of the bacteria to the active surface of the foams.

Figure 4 presents the growth inhibition of *E. coli* and *S. aureus* exposed to Ag foams made using 60, 70 and 80 vol% NaCl of various particle sizes. At equivalent volume addition of NaCl, the Ag foams made using larger NaCl particles tend to have higher antimicrobial activity for gram-negative bacteria (*E. coli*) than the foams made with smaller particles. However, effect of particle sizes and pore sizes of the foams on the bacterial growth inhibition is not as much as the effect of NaCl addition. For *S. aureus*, the highest % reduction could be found at 80 vol% NaCl addition when compared to 60 and 70 vol% addition (p value < 0.05), but this does not seem to be the case for *E. coli*.

To compare the effect of average particle size on antimicrobial activity, the Ag foams of gram-positive bacterium (*S. aureus*), do not show a similar tendency of

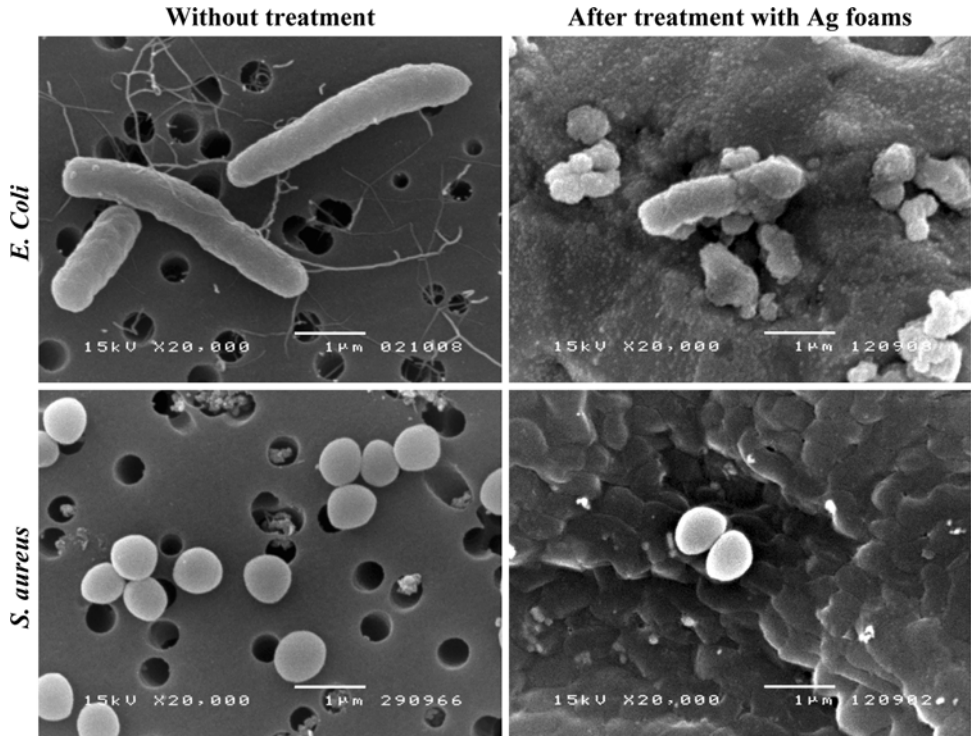

Fig. 5 Morphologies of *E. coli* and *S. aureus* without any treatment and after exposing to Ag foams made using 494 μm NaCl particles of 70 vol% for 15 min

antimicrobial activity to the *E. coli*, as expected (Fig. 4). Moreover, there was no correlation between particle sizes and bacterial growth inhibition. Repetitive tests also showed similar results. It is more likely that the bacteria were not fully exposed to internal interconnected pores of the foams. With higher resistant nature to the attack of Ag ion, due to the stronger cell wall surface of the bacteria, the growth inhibition results might be fluctuated.

In order to confirm the destructive effect of Ag foams on bacterial cells, electron microscopy was performed after treatment of bacterial cells. Figure 5 presents the morphologies of *E. coli* and *S. aureus* without treatment and after exposing to Ag foams for 15 min. Normally, rod and spherical shapes are morphology of *E. coli* and *S. aureus*, respectively. It can be seen that after exposure there were residual strains of *E. coli* and *S. aureus* on pore surfaces. However, cell surface irregularities and depressions were present, indicating a damage of the bacterial strains, especially for *E. coli*. It suggests that the Ag ion released from the surface of Ag foams can cause cell disruption, which may then result in a decrease in number of viable colonies. Similar change in bacterial morphology has been previously reported on silver nitrate and titanium dioxide nanoparticles [20, 21]. The possible mechanism of Ag ion as antibacterial agents has been explained by its ability to complex with anions such as $–NH_2$, –S–S– and –CONH– of protein or enzyme in the bacterial cells. They, therefore, can damage the DNA and RNA of bacteria or inhibit its propagation when they are released from the Ag foam in the solution [22].

4 Conclusions

Open-cell Ag foams produced through a replication process had been fabricated in this study. The foams demonstrate stronger microbial growth inhibition on gram-negative bacterium (*E. coli*) than gram-positive bacterium (*S. aureus*). Higher antimicrobial efficiency was found for Ag foams with larger pore size, at equivalent volume addition of NaCl particles. Nevertheless, the difference in the antimicrobial effects between various pore sizes of the foams is smaller when foam porosity increases from 60 to 80%. No correlation between particle sizes of NaCl and bacterial growth inhibition was found. The study shows that the effect of particle addition on the bacterial growth inhibition is stronger than the effect of particle sizes and pore sizes of the foams. The positively charged Ag ions released from the surface of Ag foam structure altered the morphology of bacteria strains in which disruption of cell wall and eventually damage were implemented.

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References

1. Brothers AH, Scheunemann R, DeFouw JD, Dunand DC. Processing and structure of open-celled amorphous metal foams. Scr Mater. 2005;52:335–9.
2. Pollien A, Scheunemann R, DeFouw JD, Dunand DC. Graded open-cell aluminium foam core sandwich beams. Mater Sci Eng A. 2005;404:9–18.
3. Nieh TG, Higashi K, Wadsworth J. Effect of cell morphology on the compressive properties of open-cell aluminum foams. Mater Sci Eng A. 2000;283(1–2):105–10.
4. Despois JF, Mortensen A. Permeability of open-pore microcellular materials. Acta Mater. 2005;53:1381–8.
5. Banhart J. Manufacture, characterisation and application of cellular metals and metal foams. Prog Mater Sci. 2001;46(6):559–632.
6. Conde Y, Despois JF, Goodall R, Marmottant A, Salvo L, San Marchi C, et al. Replication processing of highly porous materials. Adv Eng Mater. 2006;8(9):795–803.
7. Asavavisithchai S, Nisaratanaporn E. Fabrication of open-cell silver foams using a replication process. In: Lefebvre LP, Banhart J, Dunand D, editors. Porous metals and metallic foams. Pennsylvania: DEStech Publications; 2008. p. 185–8.
8. Grier N. Disinfection, sterilization and preservation. 3rd ed. Philadelphia: Lea & Febiger; 1983.
9. Russell AD, Hugo WB. Antimicrobial activity and action of silver. Prog Med Chem. 1994;31:351–70.
10. Richards RME. Antimicrobial action of silver nitrate. Microbios. 1981;31(124):83–91.
11. Liau SY, Read DC, Pugh WJ, Furr JR, Russell AD. Interaction of silver nitrate with readily identifiable groups: relationship to the antibacterial action of silver ions. Lett Appl Microbiol. 1997;25(4):279–83.
12. Cho KH, Park JE, Osaka T, Park SG. The study of antimicrobial activity and preservative effects of nanosilver ingredient. Electrochim Acta. 2005;51(5):956–60.
13. Furr JR. Antibacterial activity of Actisorb Plus, Actisorb and silver nitrate. J Hosp Infect. 1994;27(3):201–8.
14. An J, Wang D, Luo Q, Yuan X. Antimicrobial active silver nanoparticles and silver/polystyrene core-shell nanoparticles prepared in room-temperature ionic liquid. Mater Sci Eng C. 2009;29(6):1984–9.
15. Maneerung T, Tokura S, Rujiravanit R. Impregnation of silver nanoparticles into bacterial cellulose for antimicrobial wound dressing. Carbohydr Polym. 2008;72(1):43–51.
16. Ruparelia JP, Chatterjee AK, Duttagupta SP, Mukherji S. Strain specificity in antimicrobial activity of silver and copper nanoparticles. Acta Biomater. 2008;4(3):707–16.
17. Carja G, Kameshima Y, Nakajima A, Dranca C, Okada K. Nanosized silver-anionic clay matrix as nanostructured ensembles with antimicrobial activity. Int J Antimicrob Agents. 2009;34(6):534–9.
18. Slawson RM, Van Dyke MI, Lee H, Trevors JT. Germanium and silver resistance, accumulation, and toxicity in microorganisms. Plasmid. 1992;27(1):72–9.
19. Gibbins B. The antimicrobial benefits of silver and the relevance of microlattice technology. OWM. 2003;49(6):5–6.

20. Kangwansupamonkon W, Lauruengtana V, Surassmo S, Ruktanonchai U. Antibacterial effect of apatite-coated titanium dioxide for textiles applications. Nanomedicine. 2009;5(2):240–9.
21. Li Y, Leung P, Yao L, Song QW, Newton E. Antimicrobial effect of surgical masks coated with nanoparticles. J Hosp Infect. 2006;62(1):58–63.
22. Yang H, Xiao B, Xu KW. Synthesis and characterization of Ag/Cu/HAP with platelet morphology. J Mater Sci Mater Med. 2009;20:785–92.