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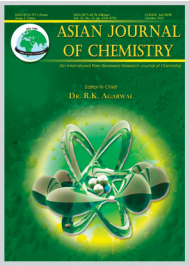

Preparation, Characterization and Antibacterial Effects of GC/PEG Nanocomposites Containing Silver Nanoparticles

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Novel nanocomposites consisting of genipin cross-linked chitosan (GC), poly(ethylene glycol) (PEG) and silver nanoparticles showed high antibacterial activity and were suitable for wound dressing. Various amounts of silver nanoparticles were added to GC/PEG matrix improve the film properties of nanocomposites. Water uptake ratios, antibacterial activity and cell culture of nanocomposite films were studied depending on the content of silver nanoparticles. With the increase of silver nanoparticles content, the water uptake ratios and the antibacterial activity of nanocomposite films were improved. Silver nanoparticles had the dual functions of uptake water and enhancing antimicrobial activity. Incorporation of silver in the GC/PEG matrix allowed the GC/PEG/silver nanocomposite to be applied successfully as the wound-healing materials.

Key Words: Chitosan, Silver, Antibacterial activity, Nanocomposite, Wound dressing.

INTRODUCTION

Despite major advances in burn wound management and other supportive care regimens, infection remains the leading cause of morbidity in the thermally injured patients and the search for different treatments and new ideas is continuing[1]. Silver metal and silver ions have been known as effective antimicrobial agents for a long time. The application of silver-binding membranes has recently been suggested to further reduce the silver toxicity, to retard the movement of silver ions and to minimize silver absorption at a healing wound[2-4].

There are many ways to incorporate silver nanoparticles into polymer matrix[5,6]. Among them, the incorporation of silver nanoparticles into films has attracted a great deal of attention, because the nanocomposites show strong antimicrobial activity. A bumber of reports have revealed that various polymer materials, such as poly(vinyl alcohol)[7,8], poly(L-lactide)[9], polyacrylonitrile[10,11], cellulose acetate[12], poly(N-vinylpyrrolidone)[13], sulfonated poly(ether ether ketone)[14] and polyimide[15], could be used as polymer matrices.

Chitosan/PEG blend films may provide additional functionality compared with the pure chitosan films. Chitosan may improve mechanical properties and decrease water solubility of the PEG films, while PEG may contribute to the formation of flexible films. Chitosan and PEG based membranes were prepared using glucose mediating process[16]. Genipin can form stable cross-linked products with enzymatic degradation resistance that is comparable to that of glutaraldehyde fixed tissue[17,18]. This study aims to investigate the feasibility of a novel nanocomposite of genipin cross-linked chitosan (GC)/PEG film in which various amounts of silver nanoparticles were embedded for the wound-dressing applications.

In this study, the antibacterial efficiency of silver nanoparticles of GC/PEG films was investigated in terms of the characteristics of GC/PEG/Ag nanocomposites. The antibacterial efficiency of the nanocomposite against *Escherichia coli* (*E. coli*) was analyzed for judging the feasibility of their use as wound dressings.

EXPERIMENTAL

Genipin and chitosan (> 85 % deacetylated) were purchased from Sigma. Silver nanoparticles (<100 nm) were obtained from Aldrich chemicals. Poly(ethylene glycol) (PEG) (m.w. 4,000) was purchased from Sigma. The buffer solutions of pH 2.0, 5.0, 7.0, 10.0 and acetic acid were purchased from Samchun Pure Chemical.

Preparation of GC/PEG nanocomposites containing silver: Aqueous chitosan solution of 1.5 % (w/v) was prepared by dissolving chitosan powder in distilled water that contained 1 % (v/v) acetic acid. Chitosan/PEG/Ag solutions were prepared by mixing chitosan solution and PEG with various amounts of silver nanoparticles. Genipin was added to chitosan/PEG/

Ag mixtures and GC/PEG/Ag nanocomposites (genipin 0.4 %, PEG 30 %). Films with a thickness of 1 mm were prepared by casting the GC/PEG/Ag mixtures onto plastic dishes. They were dried at room temperature for 48 h and then further vacuum-dried to remove any residual solvent. The films were stored in a desiccator at room temperature for further experiments. The GC/PEG films with various contents (10, 20, 50, 100 and 200 ppm) of Ag nanoparticles were labeled as GC/PEG/Ag10, GC/PEG/Ag20, GC/PEG/Ag50, GC/PEG/Ag100 and GC/PEG/Ag200, respectively.

FT-IR analysis: The Fourier transform infrared attenuated total reflectance spectroscopy (FTIR-ATR) spectra of GC/PEG, GC/PEG/Ag20 and GC/PEG/Ag50 films spectra were recorded at room temperature in the range 4000-500 cm^{-1}.

Observation of surface morphology: The morphologies of the GC/PEG/Ag nanocomposites were characterized by field emission scanning electron microscopy (FESEM). The morphology was also examined using transmission electron microscopy (TEM) of thin sections that had been prepared by casting the GC/PEG/Ag nanocomposites onto transmission electron microscopy copper grids. The samples were observed under a transmission electron microscope operated.

Water sorption studies: The water sorption capacity of each test sample was determined by immersing the sample in a capped plastic tube that was filled with 10 mL of buffered solution (pH 7) at room temperature. A known weight of each sample was placed in the medium for 4 and 24 h. The wet weight was determined by first blotting the swollen film with filter paper to remove the excess surface water and then weighing the sample immediately on an electronic balance. The water uptake ratio (WR):

$$WR = (Ws-Wd)/Wd$$

where, Ws is the weight of the swollen sample at a given time during swelling and Wd is that of the dry sample. Each swelling experiment was repeated three times and the mean value was recorded.

Cell culture: The GC/PEG/Ag nanocomposites were sterilized overnight in UV light irradiation and rinsed twice with sterile phosphate-buffered saline (PBS) and were placed into a 24-well cell culture plate well. ATCC25922 cells were cultured in Dulbecco's modified eagle medium (DMEM) that was supplemented with 10 % fetal bovine serum and 1 % (v/v) antibiotics. 1 mL of cell suspension with a density of 1×10^4 cells/mL was seeded uniformly on the surface of each test sample. The samples were cultured in a saturated humidified atmosphere of 5 % CO_2 at 37 ºC. After cultured for 6 h, SEM was used to detect the morphological variation caused by the growth of ATCC25922 cells. After each culturing period, samples were washed in phosphate-buffered saline solution for 0.5 h. After that, the samples were dehydrated using a series of ethanol solutions. The samples were dried overnight at room temperature, coated with gold by sputtering and characterized by SEM.

Antibacterial test: The *in vitro* antibacterial activity against *Escherichia coli* of GC/PEG films with various amounts of silver nanoparticles was evaluated using a cell counting method. *Escherichia coli* ATCC25922 were selected as indicators of experimental bacteria. Nutrient broth (Difco) was used as a growing medium for the microorganisms *E. coli.* Bacteria were grown aerobically in nutrient broth (Difco) at 37 ºC for 24 h. Then 50 mg of sample GC/PEG, GC/PEG/Ag10, GC/PEG/Ag50, GC/PEG/Ag100, GC/PEG/Ag200 was added to keep in contact with *E. coli*. The solution was then spread on an agar dish. The bacteria were cultured for 2 h and those in each dish were counted using the counter. The anti-bacterial ratio (R in %) of the specimen was calculated using the following equation:

$$R\ \% = (A - B)/A \times 100\ \%$$

where, A is the number of bacteria on the untreated sample (GC/PEG film) and B represents the number of bacteria on the treated sample (GC/PEG/Ag nanocomposite).

RESULTS AND DISCUSSION

As seen in Fig. 1, the increase of the silver content in the GC/PEG/Ag nanocomposite films leads to the increase in the intensity of bands at 1649 cm^{-1} and 1556 cm^{-1}. Bands are assigned to amide I(C=O) and amide II(N-H), which are the stretching vibrations of chitosan.

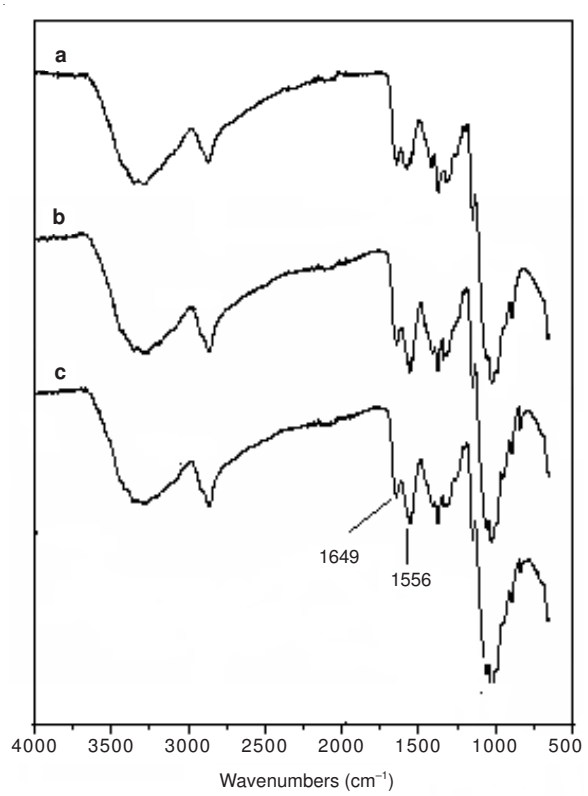

Fig. 1. FTIR spectra of GC/PEG/Ag nanocomposites with various Ag contents (a) GC/PEG, (b) GC/PEG/Ag-20 ppm and (c) GC/PEG/Ag-50 ppm

Fig. 2 presents the field emission scanning electron microscopy micrographs of the surfaces of GC/PEG film and GC/PEG/Ag nanocomposite film. The GC/PEG film showed the smooth surface morphology. Ag nanoparticles were uniformly distributed on the surface of GC/PEG/Ag nanocomposite without severe aggregation.

Fig. 3 presents the GC/PEG film and GC/PEG/Ag nanocomposite as viewed under TEM. Spherical Ag nanoparticles with diameters of about 30-90 nm were observed. The TEM image further verified that the Ag nanoparticles were distributed uniformly in the GC/Ag nanocomposite.

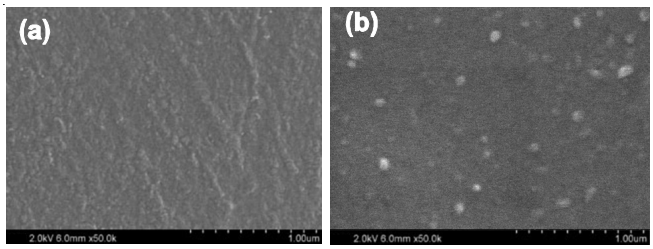

Fig. 2. Field emission scanning electron microscopy micrographs of (a) GC/PEG and (b) GC/PEG/Ag 50 nanocomposite film

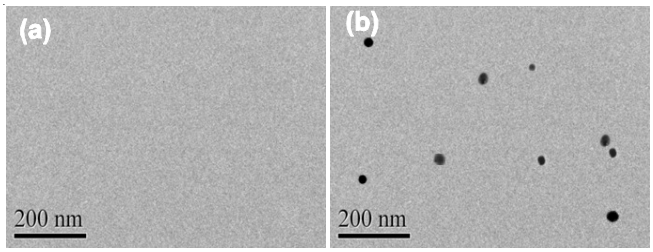

Fig. 3. TEM images of (a) GC/PEG and, (b) GC/PEG/Ag 50 nanocomposite film

Water uptake ratio: Fig. 4 presents the effect of the Ag nanoparticles on the swelling behaviour of GC/PEG films. GC/PEG film had a water uptake ratio of about 115.2 % and 120.0 % after 4 and 24 h of soaking respectively. All of the samples that were embedded with Ag nanoparticles had a significantly higher water uptake ratio than the GC/PEG film for a given soaking period ($P < 0.05$). Corresponding slight increases in the swelling of test samples with Ag content were observed. However, no significant differences in the water uptake ratio were observed among the GC/PEG/Ag nanocomposites ($P > 0.05$). The swelling kinetics indicates that the GC/PEG/Ag nanocomposites had faster swelling rates than the corresponding GC/PEG film. Adding Ag nanoparticles increased the water uptake ratio of GC/PEG/Ag nanocomposites and the swelling rates accelerated with increasing Ag content. The improvement in the water uptake ratio in the GC/PEG/Ag nanocomposites may be attributable to the presence of Ag nanoparticles in the GC/PEG matrix. The existence of Ag nanoparticles in chitosan solution affected some of the cross-linking structure in the GC/PEG matrix, reducing the crosslink density. Consequently, the GC/PEG/Ag nanocomposites swelled rapidly and achieved slightly higher swelling ratio.

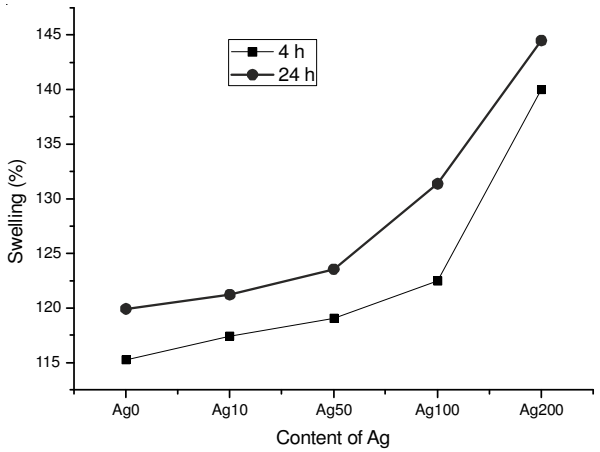

Fig. 4. Effect of Ag content on water uptake ratio of GC/PEG/Ag nanocomposites

Cells cultured: Cells on the surface of the GC/PEG film displayed a compact morphology with smooth dorsal surfaces (Fig. 5a). On the surfaces of GC/PEG/Ag nanocomposites, as revealed by the presence of significant numbers of cell microvilli. Cells were swollen and many showed damage at the end. For example, on the surfaces of GC/PEG/Ag50 nanocomposites, cell body and cell surface becoming roughness, indicating that they had adapted effectively as migration on these surfaces (Fig. 5b).

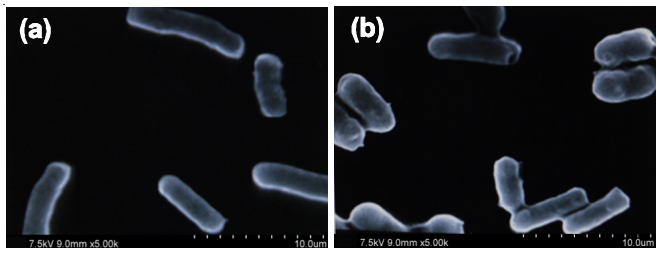

Fig. 5. SEM micrographs of ATCC25922 cells after being cultured on surfaces of (a) GC/PEG film and (b) GC/PEG/Ag 50 nanocomposites for 6 h.

Effect of Ag content on antibacterial ratio of GC/PEG/Ag nanocomposite: The Ag nanoparticles that were embedded in the GC/PEG matrix were responsible for the antibacterial reagents. Fig. 6 shows the number of viable cells of *E. coli* suspended in solution after being kept in contact with 20 mg of GC/PEG/Ag nanocomposites for several different periods. The effect of Ag content on the antibacterial activity of GC/PEG/Ag nanocomposite was clearly observed. However, the GC/PEG film without Ag nanoparticles exhibited less antibacterial activity against *E. coli*. As expected, the antibacterial effect increased with Ag content in all cases. The number of *E. coli* cells decreased to zero quickly as the Ag content increased. The antibacterial activity of GC/PEG/Ag nanocomposite was improved remarkably as the Ag content increased. *E. coli* colonies could not be observed for the GC/PEG/Ag nanocomposite containing Ag of 200 ppm. The amounts of Ag ions released from the matrices of GC/PEG/Ag nanocomposite seemed to govern their antibacterial activity. The antimicrobial properties of the Ag have been exploited for a long time in the biomedical fields. The low propensity of bacteria to develop resistance to Ag-based products allows both metallic and ionic Ag to be incorporated in biomaterials[19-21] and in medical devices for healing wounds[22].

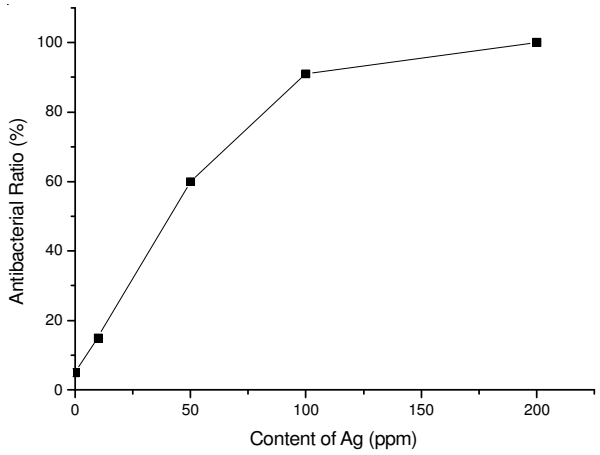

Fig. 6. Effect of Ag content on antibacterial activity of GC/PEG/Ag nanocomposites

Conclusion

The effect of Ag nanoparticles on the material characteristics and the antibacterial efficiency of GC/PEG matrix were investigated. The Ag nanoparticles were uniformly distributed in GC/PEG matrix without aggregation. With the increase of silver nanoparticles content, the water uptake ratios and the antibacterial activity of nanocomposite films were improved. Ag ions had the dual function of uptake water and enhancing antimicrobial activity.

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