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Manufacture of solid Bacteriocide Using AgNPs-SA Film and NaCl

Rachel Choi[1] and Esther Snedeker[#]

[1]Gangnam International School, Republic of Korea
[#]Advisor

ABSTRACT

When AgNO3 and sodium alginate are mixed, cross-linking occurs by the cation Ag+ and anion Cl-, which can produce an AgNPs-SA gel. Through this research, it was found out that when this film is treated with NA or NB, the film decomposes. Therefore, we investigated the cause of this phenomenon and found a way to utilize it. As a result, the AgNPs film produced by the reaction of 0.1 M AgNO3 and 2% SA solution visibly disappeared and decomposed by NB after 30 minutes. It was thought that the cause was NaCl, a salt contained in beef extract or peptone, the ingredients for NB. The AgNPs-SA gel and film decomposed within 0.01 M to 1 M of NaCl solutions, and it seemed that Ag+, which plays role in cross-linking, reacted with NaCl to form AgCl, which caused the bond to decompose. When the film solution dissolved in 0.01 M to 1 M of NaCl solution for 30 minutes was treated with E. coli and S. aureus, the bacterial inhibition effect was the best in the solution decomposed in 0.01 M, 0.05 M, and 0.1 M of NaCl solutions. As a result, in this research it was found that the AgNPs-SA gel could be a solid antibacterial film that is possible to store in film form and, when used, a part of the film can be put into NaCl solution to make a liquid disinfectant, which can help patients with alcohol allergies and can be applied in places such as livestock farms affected by foot-and-mouth disease.

Introduction

The antibacterial effect of silver ions can suppress microorganisms through various processes, including inactivation of microorganisms through binding of silver ions to proteins within microorganisms, generation of active oxygen during the metabolic process of microorganisms, inhibition of the production of K+ ions in the cytoplasmic membrane of microorganisms, and direct effects on DNA [1].

An eco-friendly method of making silver nanoparticles is known to be to use a technology that reduces silver ions using components contained in plant or fruit peel extracts to make nanoparticles [2]. Using the same principle, a hydrogel with silver nanoparticles can be made by treating sodium alginate, known as a polysaccharide contained in seaweeds such as kelp, with silver nitrate. Since silver ions are cross-linked with alginate and silver is reduced, a hydrogel with AgNPs formed can be easily obtained. Therefore, research is being conducted on a sterilizing film that mixes silver ions, sodium alginate, agar, etc. using this method [2. 3].

Referring to the above data, a film was made by reacting AgNO3 and SA (Sodium Alginate), and an in-school exploratory experiment on the antibacterial effect was conducted. When treated with NA (Nutrient Agar), the SA film on which AgNPs were formed was dissolved on the NA medium as shown in Fig. 1. C), and when treated with NB, it was also found to decompose quickly. The film made with 1% agar, which was the comparative target, did not decompose.

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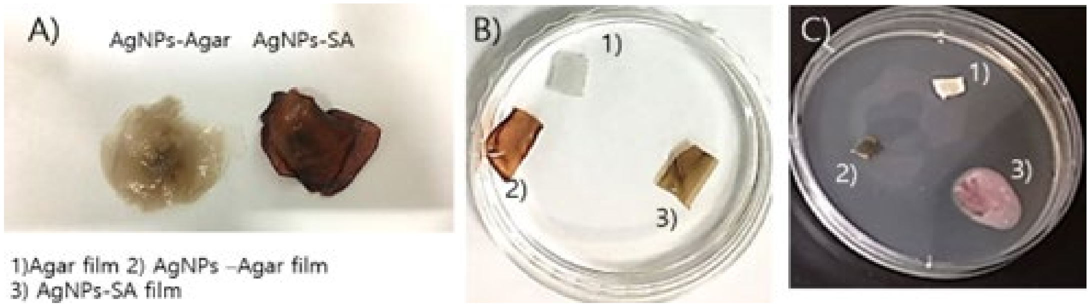

Figure 1. A) AgNPs film made by treating AgNO3 on agar and sodium alginate B) When the film made in A is placed in water; C) When the film made in A is placed on NA, the change in the film when placed in water and NA medium is shown in 3) Only the film is dissolved in the NA medium.

The reason for the above phenomenon can be thought of as peptone and beef extract, which consists NA and NB. The The main ingredient of peptone is inorganic salts [4], and beef extract also contains salt, for less than 7% [5]. Therefore, it was assumed that this salt component was the cause of dissolving the AgNPs sodium alginate film. This is because the salt contained here can cause a reaction between the chloride ion of NaCl and the Ag ion that participated in the cross-linking of the sodium alginate, thereby breaking the cross-linking of the hydrogel, and when a hydrogel capsule is made with AgNO3 and sodium alginate, the chloride ion of sodium chloride can form a precipitate and collapse the capsule [6]. The AgCl formed at this time When AgNO3 is reacted with plant extracts, silver ions are reduced, and when chlorine ions are added at this time, AgCl with antibacterial properties is generated [2]. AgCl has antibacterial properties similar to AgNPs, so when forming AgNPs, it is reacted with ZnCl2 to enhance the antibacterial properties of AgNPs [7].

On the other hand, when looking at the relationship between AgNPs and salt, it is said that although the capsules can be collapsed, in the presence of organic substances such as seawater, sodium chloride, tannin, and alginic acid, the phenomenon of Ag ions clumping can also occur [8]. Therefore, we investigated the cause of the decomposition of the hydrogel formed with AgNPs in NA and NB, made the AgNPs hydrogel into a film, and reacted this film with NaCl to determine whether the collapsed solution could be used as a disinfectant.

Method

Fabrication of Ag-SA (Ag-Sodium Alginate) Film

We decided to make a hydrogel by cross-linking alginate and silver nitrate and then dry the gel to make a film. First, we tried to find the concentration suitable for gel formation by varying the concentration of silver nitrate in sodium alginate. 3 mL each of AgNO3 prepared at concentrations of 0.0125 M, 0.025 M, 0.05 M, and 0.1 M was added to 7 mL of 2% sodium alginate solution in a petri dish. Afterwards, the petri dish was placed on a slide warmer set to 60°C to allow AgNPs to form well, and the gel was dried to create an Ag-SA film.

Changes In Alginate Film and Antibacterial Effect According to NB Treatment

NB 100% was made by adding 8g of NB powder to 1L and sterilizing at 121℃ for 15 minutes. This was diluted again to make a NB 50% solution. Then, 5 mL of 50% and 100% NB solutions were placed in petri dishes, and the film made in 2.1 was cut and placed together. Changes were checked for 30 minutes and after 1 day.

And to confirm the reaction between 1 mL of NB solution and silver ion, 1 mL of 0.1 M AgNO3 solution was added together, and the precipitation reaction was observed. Finally, each 1 and 2 pieces of film (approximately

0.5 cm x 0.5 cm) were added to 1 mL of NB solution, respectively, and 100 µL of E. coli and S. aureus solutions were added. After 30 minutes, 40 uL of the solution was collected, spread on NA medium, and treated with 1 mL of NB. After 1 day, the bacterial communities of E. coli and S. areus grown on the NA medium were confirmed and the absorbance of the NB solution was measured.

Reaction and Antibacterial Effect of Ag-SA Gel and Film Treated with Nacl According to Concentration

Since it was confirmed that Ag-SA film was dissolved in NB, the cause was judged to be NaCl contained in NB, and the possibility of using a disinfectant dissolved in NaCl solution was investigated. To this end, we tried placing the AgNPs-SA gel and the film made by drying the gel into a NaCl solution. The AgNps-SA gel was made to have a diameter of approximately 8 mm in the gel state, as shown in the figure below. And the film was made to have a weight of approximately 0.26 g after drying.

Figure 2. Ag-Sa gel and film formed by mixing AgNO3 and sodium alginate.

After putting this gel and film into NaCl solution at 0.01M, 0.025M, 0.05M, 0.1M, 0.5M, and 1M. The degree of decomposition was confirmed according to time and concentration. Then, 20 µL of E. coli and S. aureus and 20 µL of the solution containing the film were treated on NA medium, spread, and cultured. The ratio of the total surface area occupied by the bacterial colony was analyzed using Image J.

Experimental Results

Hydrogel Formation According to The Concentration of AgNO3 and SA

When AgNO3 was added to SA and mixed, it was confirmed that the color immediately changed to white. The reason for this appears to be that the Ag+ ions were cross-linked with sodium alginate.

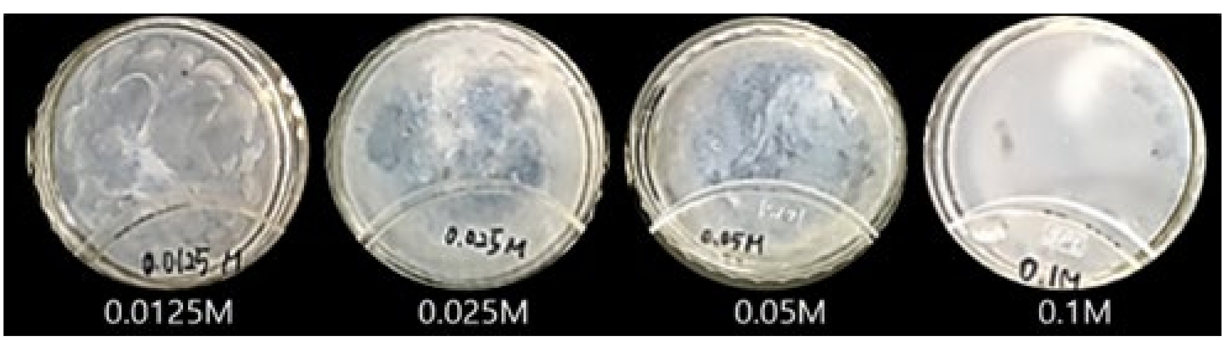

Figure 3. 0.012M~ 0.1M AgNO3 and sodium alginate were mixed.

In addition, to speed up the reaction, it was placed on a 60-degree Celsius slide warmer. As time passed, the side with lower silver nitrate concentration began to turn gray. and the side with higher silver nitrate concentration began to turn brown.

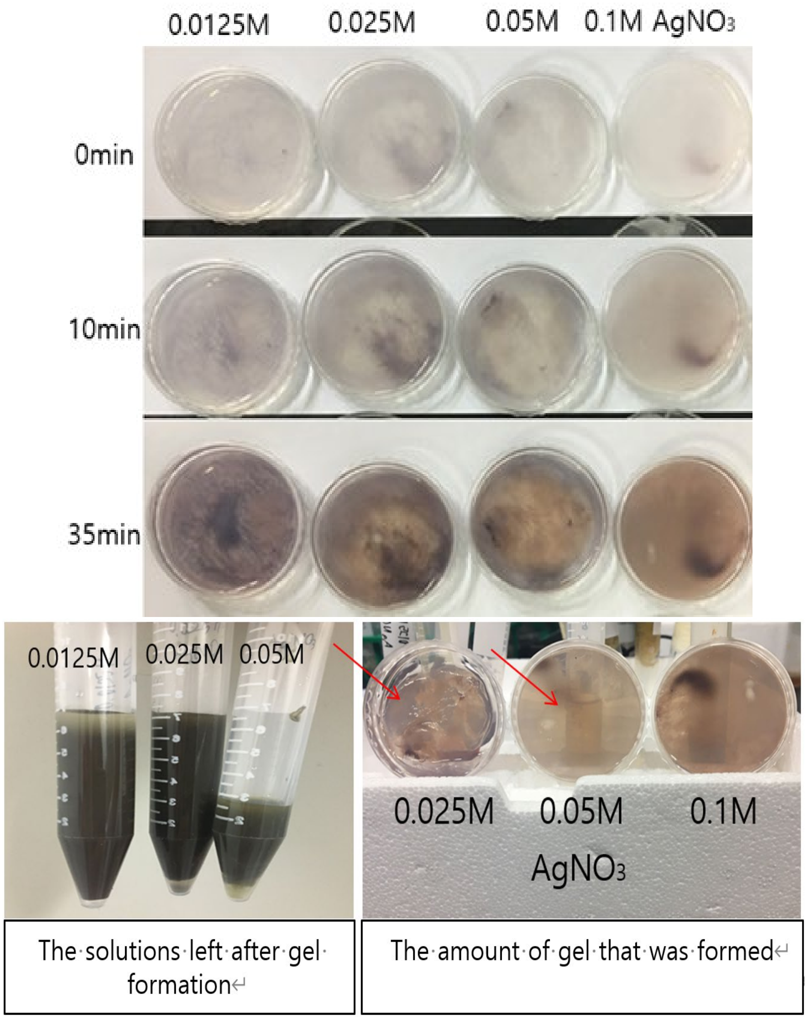

Figure 4. Changes in SA hydrogel according to AgNO3 concentration

Hydrogel made by concentration of 0.0125M ~ 0.05M AgNO3 changed partially into a solution with the rest by gel, with the remaining solution decreasing by higher concentration. Only the 0.1M concentration formed an absolute gel, with no solutions remaining after formation. It appears that the solution color changed to dark green because the SA hydrogel was not formed and therefore reacted with AgNO3 in a sol state, making AgNPs exist in a solution form. It appears that in cases where the gel was relatively well formed, the Ag+ ions participated in cross-linking to form AgNPs, so it appeared brown. The best gel formation was achieved by mixing 0.1 M AgNO3 and 2% sodium alginate in a ratio of 3:7, and a film was formed under these conditions.

Decomposition Of Alginate Film According to NB Solution Treatment

After adding AgNO3 to the NB solution, we checked whether a precipitation reaction occurred. After 5 minutes, a yellow precipitate appeared in the NB solution. And after 1 day, a brown precipitate appeared.

The yellow precipitation appears to be a reaction between AgNO3 and the salt contained in NB, and the brown precipitation is expected to be a reduction of some of the Ag+ ions by the protein component contained in NB.

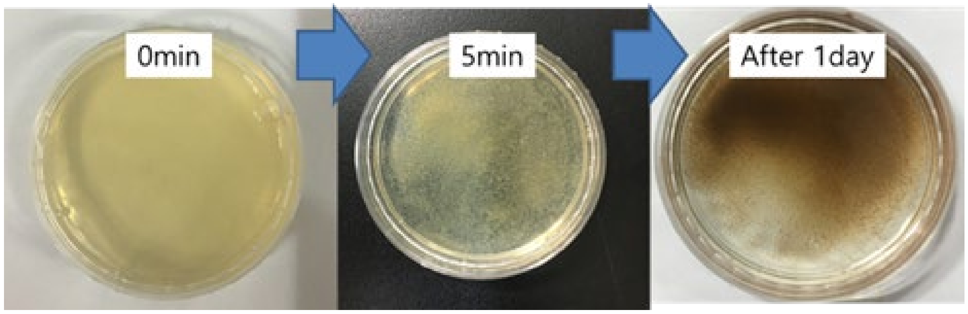

Figure 5. Reaction of NB solution and AgNO3 solution

The changes in the pieces placed in 0%, 50%, and 100% NB solutions for 0, 10, 20, and 30 minutes were observed. It was observed that the higher the NB concentration, the more the shape of the piece that was treated for a long time was decomposed. On the other hand, the piece placed in water did not decompose but only swelled as it absorbed water little by little. Therefore, it could be concluded that the salt in the NB solution affected the decomposition of the AgNPs-SA film.

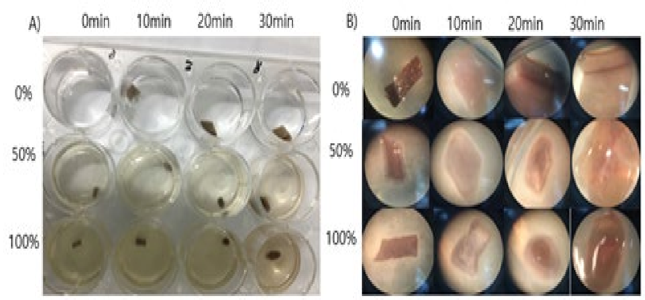

Figure 6. A) AgNPs-SA film placed in NB solution. B) Changes in the film when AgNPs-SA film was treated in 0%, 50%, and 100% NB solutions for 10, 20, and 30 minutes.

The film that had been placed for 0 to 30 minutes was left at room temperature for a day, and the changes in the film were observed. As a result, the film in the 50% and 100% NB solutions dissolved, making it difficult to find the shape of the pieces compared to the film that showed almost no change in the 0% solution.

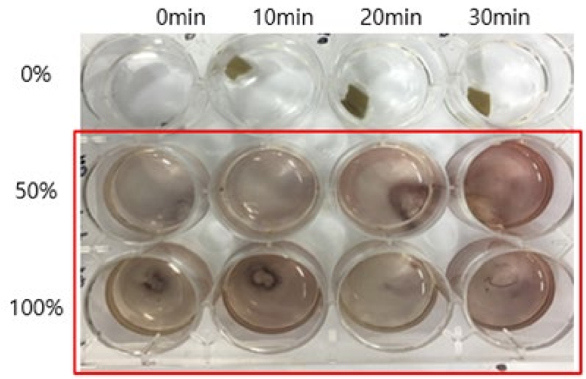

Figure 7. Changes in AgNPs-SA film reacted with solutions containing Cl ions.

In order to confirm the sterilizing power, 1 and 2 pieces of the pieces from the above experiment were put in, respectively, and the decomposed solution was cultured in E. coli and S. aureus. As a result, both E. coli and S. aureus showed a 10-fold decrease in bacterial absorbance when treated with solutions containing 1 and 2 pieces of film, respectively, compared to the control group. When confirmed by spreading on NA medium, E. coli and S. aureus spread and grew throughout the NA medium in the control group, but when the solution with the film pieces was put in, hardly any bacterial colonies were observed in E. coli, and a tendency for bacterial colonies to decrease compared to the control group was observed in S. aureus.

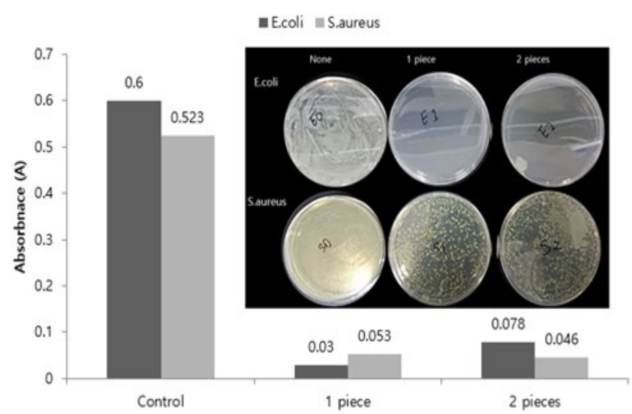

Figure 8. Absorbance when the solution in which the film was placed for 30 minutes was placed in NB medium and changes in bacterial population when this solution was spread on NA medium

Response of AgNPs-SA Gel and Film Treated with NaCl According to Concentration

It was confirmed that the salt contained in NB of 3.2 affected the gel state. Therefore, it was decided to observe the change by reacting with NaCl. When Ag-SA in a gel state was reacted with NaCl for 5 minutes, it was confirmed that the gel was completely dissolved in all concentrations of NaCl except for 0.01 M NaCl, and they were shown white or pink.

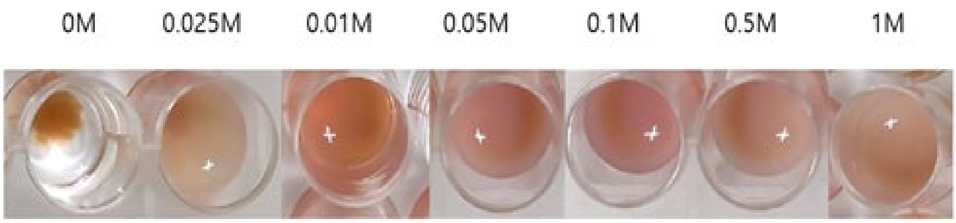

Figure 9. Changes in AgNPs-SA gel according to NaCl concentration

The AgNPs-SA film rapidly dissolved in a 0.01 M to 0.1 M NaCl solution, and the color of the dissolved substance changed to light purple. This is believed to be due to the reaction between the Ag+ and Cl- ions contained in the AgNps-SA. However, contrary to the expectation that the film would dissolve more quickly as the concentration increased and the Ag+ ions participating in the SA cross-linking escaped due to the increase in chloride ions, the dissolution rate decreased at 0.5 M and 1 M. This is believed to be due to the aggregation of AgNPs in the presence of decomposed alginate and high concentrations of NaCl. However, this phenomenon was temporary, and the film eventually dissolved over time.

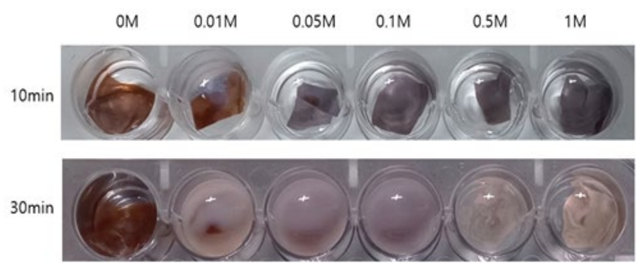

Figure 10. Changes in AgNPs-SA film according to changes in NaCl concentration

Antibacterial Effect of AgNps-SA Solution Treated with NaCl According to Concentration

The gel and film solutions reacted in 3.3 were mixed with E. coli and S. aureus and then spread on NA medium to examine the bacterial inhibition effect on the solution dissolved in NaCl. As a result of the experiment, in the case of E. coli, the bacterial colonies were observed to be very small on the side treated with the film solution dissolved in 0.01 to 0.1 M NaCl solution, and the area occupied by the bacteria was also observed to be small, about 3 to 4%. On the other hand, in the cases of 0.5 M and 1 M, the bacteria suddenly increased, and the area occupied by the bacteria was measured to be similar to the control group. In the case of S. aureus, fewer bacteria were observed at both 0.01 M and 1 M, and although the area occupied by bacteria increased at 0.5 M and 1 M, it was twice less than the control group.

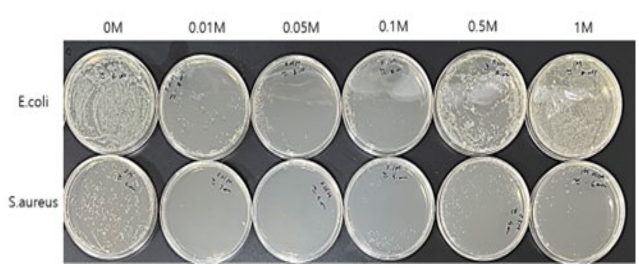

Figure 11. AgNPs-SA films were dissolved in NaCl solutions prepared at different concentrations and then treated with E. coli and S. aureus to culture the bacterial colonies.

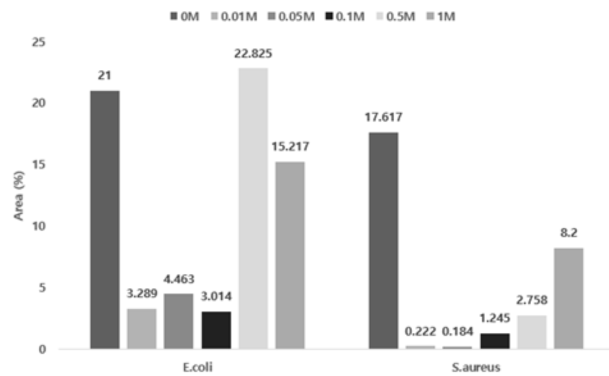

Figure 12. Area ratio occupied by E. coli and S. aureus cultured in NA medium

For this reason, the dissolution rate of the film was slow at 0.5 M and 1 M, so the antibacterial effect seemed to have decreased because fewer Ag+ ions escaped.

Discussion and Conclusion

A study was conducted to create a hydrogel using cross-linking of Ag+ and sodium alginate and to create an antibacterial film using this to create a skin disinfection film. Then, the AgNPs-SA film was treated with NA and NB to observe the antibacterial effect, and the film was observed to decompose. It was expected that the cause of the decomposition would be the salt contained in beef extract or peptone. To confirm this, 0.1 M AgNO3 was treated with NB solution, and as a result, a brown precipitate was generated, and the AgNPs film created by the reaction of 0.1 M AgNO3 and 2% SA solution decomposed after 30 minutes as the film shape disappeared noticeably. After 1 day, it was confirmed that it was completely mixed with the solution.

We thought that the cause of the decomposition was NaCl, so we placed the AgNPs-SA gel in a 0.01 M to 1 M NaCl solution, and it decomposed in 10 minutes. In addition, when the film was placed, the film changed from brown to purple after 10 minutes and was completely decomposed after 30 minutes. It seems that the cause of the decomposition was that the Ag+ ions that cross-link SA reacted with NaCl to form AgCl, and the cross-linking structure collapsed in the process. Although the film was decomposed at 0.5 M and 1 M NaCl, it was observed to dissolve more slowly than at lower concentrations. It seems that the reason for this is that AgNPs are easily agglomerated in the presence of high concentrations of NaCl and organic substances such as alginic acid, and this phenomenon slowed down the dissolution a little. When the dissolved film solution was treated with E. coli and S. aureus for 30 minutes, the bacterial inhibition effect was the best in the solution decomposed in 0.01 M, 0.05 M, and 0.1 M NaCl solutions.

Therefore, this AgNPs-SA film or gel is well decomposed by a 0.01 M to 0.05 M NaCl solution, and bacteria can be inhibited by the AgNPs and AgCl contained in the solution. Disinfectants that are usually in the form of solutions are bulky and difficult to store and require containers to hold the solutions. The use of these containers leads to an increase in plastic waste. However, if an AgNPs-SA film is made, dissolved in a NaCl solution, and then diluted to a certain amount for use as a disinfectant, not only is the disinfectant easy to store, but a disinfectant container is also unnecessary. This disinfectant can be used to help patients who have difficulty disinfecting with alcohol due to alcohol allergies or to disinfect boots when entering and exiting livestock sheds during outbreaks of foot-and-mouth disease and swine fever. It seems that it will have the advantage of lowering the freezing point due to NaCl when used in the winter.

Acknowledgments

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