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Original Article

Silver Nanoparticles Incorporated Chitosan Hydrogel as a Potential Dressing Material for Diabetic Wound Healing in Nursing Care

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ABSTRACT

Background: Nursing care of diabetic wounds remains a significant challenge due to a variety of reasons including low blood flow, impaired activity of inflammatory responses, and high oxidative stress in the diabetic wounds bed. **Materials and Methods:** In the current research, silver Nanoparticles (AgNPs) were loaded into a chitosan hydrogel system to produce a potential wound dressing for treating diabetic wounds. Various *in vitro* studies such as scanning electron microscopy assay, cell viability assay, and swelling assay were performed in order to characterize the dressing system. Wound healing potential of the developed wound dressing was investigated in a rat model of excisional wound. **Results:** *In vitro* study confirmed the biocompatibility of these hydrogels. *In vivo* study showed that wounds treated with AgNPs/chitosan hydrogel had higher wound closure and histological restoration compared with other groups. Degree of inflammation and formation of epithelium in AgNPs-loaded hydrogels were improved compared with other groups. **Conclusion:** Our developed systems have potential applicability to be used as wound dressing in nursing care.

Keywords: Wound nursing care, Silver nanoparticles, Chitosan hydrogel, Drug delivery, Wound dressing.

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INTRODUCTION

Diabetic wounds encompass various types, each with distinct characteristics. Neuropathic ulcers result from nerve damage and often occur on the feet due to reduced sensation. Ischemic ulcers develop from poor blood circulation and appear on the lower extremities. Diabetic blisters form on the extremities and may burst, leading to ulcers. Foot infections are common in diabetics and can rapidly worsen. These wounds are slow to heal, prone to infection, and require specialized care to prevent complications in individuals with diabetes.[1-3] Nursing care of diabetic wounds remains a significant challenge due to a variety of reasons including low blood flow, impaired activity of inflammatory responses, and high oxidative stress in the diabetic wounds bed.[4-6] In these patients, wound healing response is compromised, delaying the phases of wound healing and causing a chronic non-healing wound. In this condition, wound care materials play a fundamental role in closing the wound and preventing the further complications. In this context, various formulations of wound dressings such as fibers, sponges, membranes, hydrogels, and a cellular tissues have been investigated in nursing care of diabetic wounds.[7,8] Recently, chitosan-based wound dressings have gained significant attention. This polymer is a natural polysaccharide, derived from deacetylation of chitin. Chitosan promotes different phases of wound healing through its antibacterial properties, anti-inflammatory function, and proliferative activities.[9,10] Particularly, chitosan-based hydrogels are promising wound dressings that can easily fill the deep cavity of diabetic wounds and possess a high similarity to native skin tissue's extracellular matrix.[11] However, the complex pathophysiology of diabetic wounds requires a versatile strategy to combat the underlying contributing factors. Indeed, bacterial infection is one of the implicated factors in delayed wound healing in diabetic patients.[1,12,13] Therefore, the antibacterial wound healing materials are of paramount importance in nursing care of diabetic wounds. In this regard, silver Nanoparticles (AgNPs) have been widely explored for diabetic wounds applications.[14] These particles prevent bacterial infection and can also trigger wound re-epithelialization by increasing the migration potential of skin cells.[15] Based on this knowledge and principles, we aimed

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to develop an antibacterial wound dressing for nursing care of diabetic wounds.

MATERIALS AND METHODS

Preparation of chitosan hydrogels loaded with AgNPs

To begin, chitosan (high molecular weight, sourced from Sigma Aldrich, USA) was dissolved in 1% v/v acetic acid (obtained from Merck, Germany) in distilled water, reaching a final concentration of 2 wt.%. Subsequently, a suspension of AgNPs (0.2 mg/mL) was introduced into the chitosan solution at a volume ratio of 7.5% v/v, thoroughly mixed. Lastly, the pH of the solution was carefully adjusted to 7 through the gradual addition of sodium hydroxide.

Scanning Electron Microscopy (SEM)

Hydrogels with or without AgNPs were freeze dried at -80°C for 48 hr. Then, their microstructure was analyzed under an SEM device under 26 kV accelerating voltage and sputter coating with gold for 250 sec.

Swelling assay

Swelling of chitosan hydrogels with and without AgNPs incorporation was assessed by immersing 200 mg (W0) of the dry scaffolds into 15 mL PBS and keeping for 48 hr. At different time steps, scaffolds were taken out and their wet weight was measured (W1). Following equation was used for the calculation:

$$\text{Swelling percentage } (\%) = \left(\frac{W1-W0}{W0}\right) \times 100$$

MTT assay

The biocompatibility of the developed hydrogel system was assessed on L929 fibroblast cells using the MTT assay. Briefly, 10000 cells were seeded on each scaffold in 96-well plates and cultured for 7 days. On days, 1, 3, and 7 cell viability was assessed using an MTT assay kit.

In vivo study

Animal studies were performed in accordance with the university guiltiness and approved by the ethics committee of the university. Wound healing function of chitosan hydrogel and chitosan/AgNPs hydrogel was investigated in a rat model of diabetic wound. Firstly, male Wistar rats were administered with STZ for 7 days until their fasting blood sugar levels reached above 250 mg/dL. In this experimental setup, a total of twelve diabetic rats were selected for the study. These rats were allocated into three separate groups in a random manner, ensuring that each group had an equal chance of receiving any specific treatment or condition. Study groups were as follows: 1-chitosan hydrogel, 2-chitosan/AgNPs group, 3-sterile gauze group as negative control, and 4-MEDIHONEY® Gel-treated wounds as the positive control. The surgical procedure was initiated by intraperitoneal injection of ketamine and Xylazine. Then, a 1.5×1.5 cm^2 of the dorsal skin was excised after proper shaving and disinfecting. Finally, the developed hydrogels were applied on the wound tissue. The wound dressings were replaced every two days. On day 7 and 14, the wound site was imaged and the images were used for the calculation of wound size reduction.

Histopathological studies

On day 14, the animals were sacrificed; the wound tissues were processed, and stained with H&E. Histopathological images were interpreted by an independent pathologist which was blind to the study.

Statistical analysis

Data was analyzed using GraphPad prism version 5. Student's *t*-test and one-way ANOVA were utilized for statistical analysis. Data is expressed as mean±SD.

RESULTS AND DISCUSSION

SEM imaging showed that the chitosan/AgNPs and chitosan hydrogels were porous

Results (Figure 1) showed that both chitosan/AgNPs and chitosan hydrogels were highly porous in their microstructure. However, the porosity of the hydrogel systems is considerable smaller than the lyophilized scaffolds.[16] The pores in the freeze dried scaffolds could be due to the evaporation of solvent-rich phase during the freeze drying process. The pore size in these scaffolds can be enlarged through decreasing the cooling pace.[17]

Swelling study showed a two-phase swelling behaviour

Results (Figure 2) showed that chitosan/AgNPs and chitosan hydrogels could absorb water up to 496.99±33.28 and 444.50±28.53 of their weight at the 4th hr of their immersion in PBS. Then, the swelling activity of the scaffolds gradually decreased during the 48 hr study. Chitosan-based wound dressings are highly hydrophilic because of their free amine groups.[10] This property of chitosan-based hydrogels may potentially be beneficial in nursing care of chronic wounds. However, we expect that hydrogel-based systems have lower exudates absorption capacity than the lyophilized wound care materials.[18]

MTT assay confirmed the biocompatibility of chitosan/AgNPs and chitosan hydrogels

MTT assay (Figure 3) showed that on none of the studied time steps OD values in chitosan/AgNPs, chitosan, and control groups were not statistically significant, p-value > 0.05. Indeed, AgNPs and chitosan are highly biocompatible and their safe clinical applications have been shown in nursing care of chronic wounds.[14,19]

In vivo study

Wound closure study (Figure 4) showed that on day 14, AGNPs-incorporated hydrogels had significantly higher percentage of wound size reduction compared with other groups, *p*-value<0.05. Chitosan-only group did not have significantly different size reduction compared with the negative control group, *p*-value>0.05. Positive control group had significantly higher percentage of wound closure than negative control and chitosan-only hydrogel groups, *p*-value<0.05. The higher wound closure activity of chitosan/AgNPs hydrogel compared with chitosan-only hydrogel could be due to the antibacterial activity of AgNPs. In this regard, Gupta *et al*. showed that AgNPs loaded in a bacterial cellulose-based hydrogel system prevented the growth of Staphylococcus aureus, Pseudomonas aeruginosa, and Candida auris. As these bacteria are common causes of wound inflammation, AgNPs-loaded chitosan hydrogels may potentially be utilized for nursing care of infectious wounds.[20] In addition, El-Aassar *et al*. developed an AgNPs-loaded polymeric wound dressing for treating multidrug resistant bacteria-infected wounds.[21]

Histopathological studies (Figure 5) revealed remarkable observations regarding the wounds treated with chitosan-AgNPs hydrogels and MEDIHONEY® Gel. These innovative treatment modalities demonstrated not only significantly fewer inflammatory cells but also a striking improvement in the organization of collagen fibers within the wound tissue. One of the most striking findings was the enhanced formation of granulation tissue and the acceleration of epithelization in wounds subjected to these treatments, in stark contrast to the other groups. Granulation tissue, a key element in wound healing, appeared to proliferate more efficiently, contributing to a more rapid healing process. Epithelization, the crucial step where new epithelial cells cover the wound, displayed a remarkable advancement in these two groups, which translated to faster wound closure and re-epithelialization. These findings underscore the potential of chitosan-AgNPs hydrogels and MEDIHONEY® Gel as highly effective wound management options, not only in reducing inflammation and promoting collagen organization but also in significantly expediting the critical stages of granulation tissue formation and epithelization.

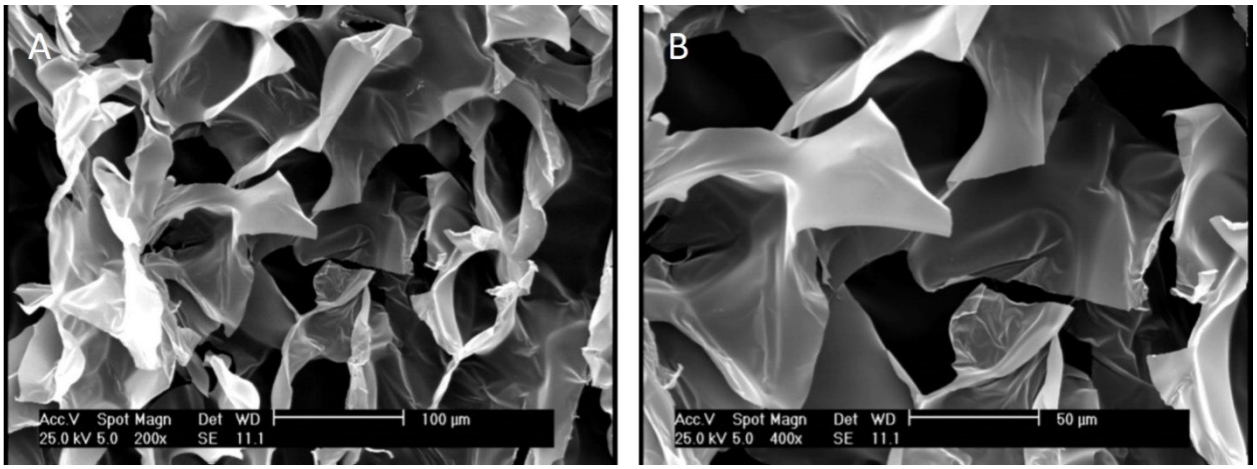

Figure 1: SEM images of (A) chitosan/AgNPs hydrogel and (B) chitosan-only hydrogels after freeze drying, scale bar shows 50 µm.

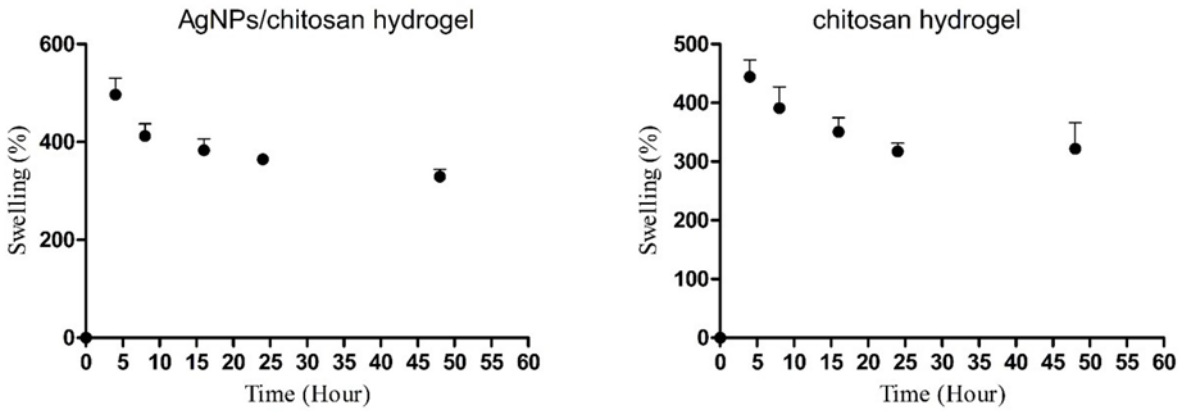

Figure 2: Swelling assay with AgNPs/chitosan hydrogel and chitosan-only hydrogel.

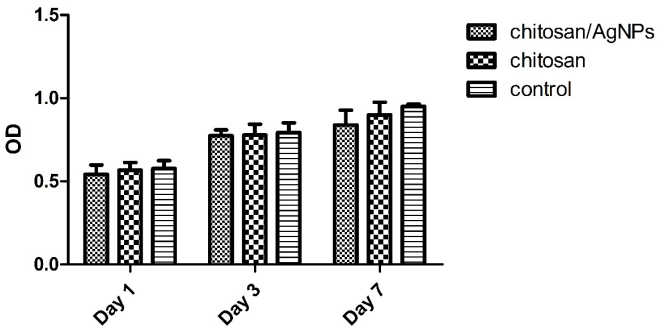

Figure 3: MTT assay with fibroblast cells cultured on chitosan/AgNPs and chitosan hydrogel on days 1, 3, and 7.

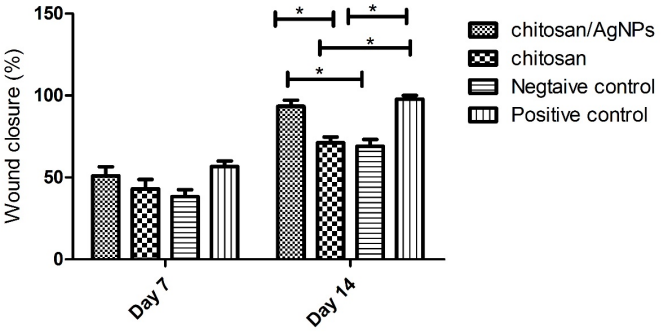

Figure 4: Wound closure assessment of rats treated with different hydrogels on days 7 and 14. *shows *p*-value<0.05.

Histomorphometric analysis (Figure 5) revealed that thickness of epithelium in wounds treated with chitosan-AgNPs hydrogels and MEDIHONEY® Gel was significantly higher than negative control and chitosan-only hydrogel groups, p-value < 0.05. It could be that immune modulatory activity of chitosan may have reduced the inflammatory cells in the wound site. This theory is in accordance with the findings of Chang *et al.*[22] They showed that contrary to low molecular weight chitosan, high molecular weight chitosan decreased the secretion of TNF-a and IL-6 levels, implying that higher molecular weight chitosan are more suitable for treating chronic wounds where hyperactivity of inflammatory responses deter the normal wound healing phases. Chitosan hydrogel loaded with AgNPs has been found to have excellent wound healing qualities, lower bacterial counts, and enhanced production of growth factors.[23] Nesovic *et al.* showed that chitosan-based hydrogel wound dressings with electrochemically incorporated silver nanoparticles provided protection and support to the wound during the entire remodelling and healing process.[24] Chitosan also plays a pivotal role in promoting collagen synthesis and alignment, fostering the organization of collagen fibers within the wound tissue. Additionally, these hydrogels stimulate the formation of granulation tissue, providing a scaffold for blood vessel growth, collagen deposition, and cell migration.[10,18] This aids in the reconstruction of damaged tissue. In addition, AgNPs actively combat wound infections, reducing the microbial load and creating a more conducive environment

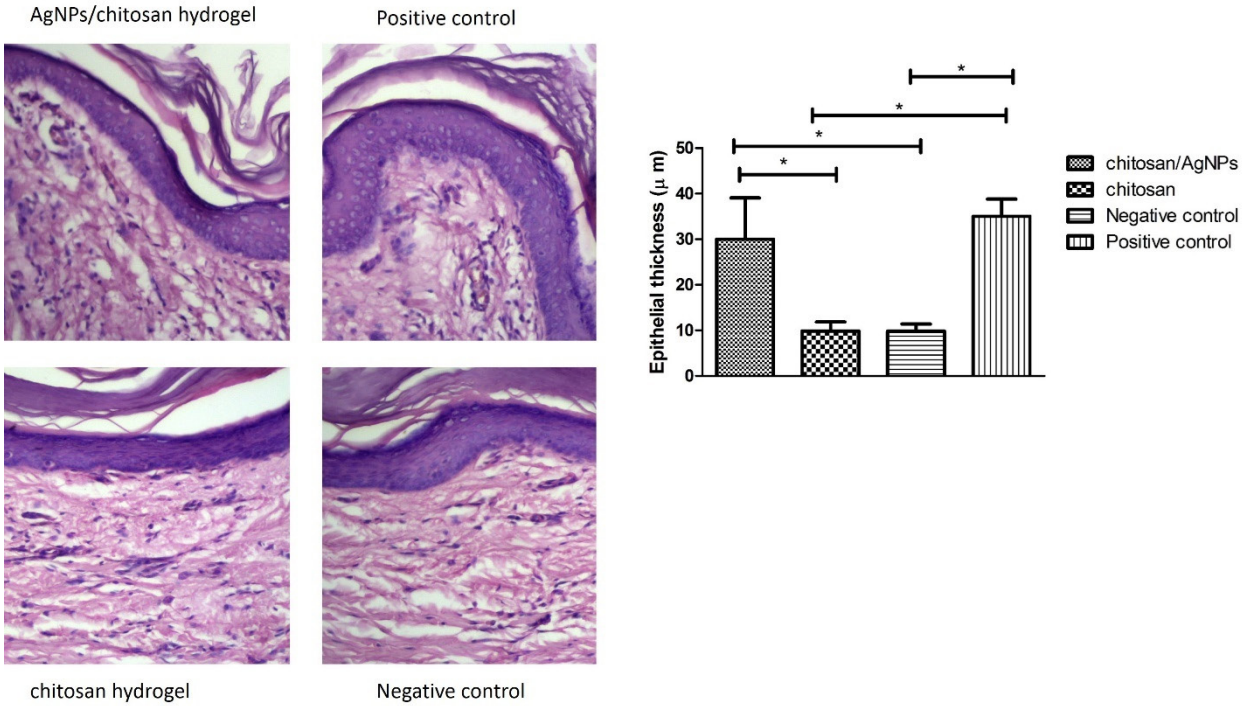

Figure 5: Histopathological images of wounds treated with hydrogels compared with control group. Obtained by H&E staining at the end of 14th day post-surgery, histogram demonstrates thickness of epithelium in wounds treated with different treatment options.

for the healing process. Importantly, chitosan-AgNPs hydrogels expedite the critical process of epithelization, which is essential for wound closure, reducing the risk of infection and scarring.[14] Their ability to maintain a moist wound environment, facilitate the controlled release of bioactive agents, further underscores their potential to promote wound healing.[25]

Limitations of this research include the need for human clinical trials and longer-term assessments for practical application as a wound dressing. Researchers may have preconceived notions or preferences for a specific outcome due to their involvement in the development of the dressing, potentially influencing the study's design and interpretation.

CONCLUSION

In the current study, silver nanoparticles were loaded into a chitosan hydrogel in order to produce an antibacterial dressing material in nursing care of diabetic wounds. *In vitro* studies showed that loading the nanoparticles into the hydrogel system did not alter their biocompatibility and swelling behavior. *In vivo* study showed that silver nanoparticle-loaded hydrogels significantly increased the wound reduction percentage in diabetic rats. This preclinical research suggests potential use of this dressing material for nursing care of diabetic wounds in the clinic. Limitations of this research include the need for human clinical trials and longer-term assessments for practical application as a wound dressing. A prospective research direction for this project could involve investigating the long-term safety and efficacy of the AgNPs/chitosan hydrogel wound dressing in a clinical setting. A randomized controlled trial with diabetic patients could assess wound healing outcomes, infection rates, and potential side effects over an extended period, providing valuable insights for practical use in nursing care.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

AgNPs: Silver Nanoparticles; **SEM:** Scanning Electron Microscopy; **STZ:** Streptozotocin; **PBS:** Phosphate buffer saline; **OD:** Optical density; **TNF-a:** Tumor necrosis factor-a.

SUMMARY

Developing antibacterial wound dressings is of vital importance in nursing care of skin wounds. Silver nanoparticles and chitosan have both antibacterial activities. In this research, we showed that incorporation of silver nanoparticles into the matrix of chitosan hydrogels significantly augmented their ability for treating skin injuries in a rat model. Therefore, our developed hydrogel have potential applicability to care skin wounds

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