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Efficacy of Novel Blue Silver Nanoparticles Hydrogel versus Reference Hydrogel: a Prospective Randomized Controlled Trial for Acute and Chronic Wound Management

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ABSTRACT

OBJECTIVE: Hydrogel dressings are commonly used as wound dressings and provide a moist environment in the wound area. Silver nanoparticles (AgNPs) are widely considered as useful therapeutic agents for the prevention and eradication of wound colonization by microorganisms. Recently, there has been a hydrogel dressing that consists of carboxymethyl cellulose hydrogel with blue AgNPs. It provides a moist and optimal healing environment for pain relief and protection from infection. This study aimed to evaluate the use of blue AgNPs hydrogel in acute and chronic wound care.

METHODS: From September 2017 to September 2018, 62 wound sites from 39 patients were randomized to receive daily application of either the blue AgNPs hydrogel (31 wound sites from 20 patients) or a commercially available reference hydrogel (31 wound sites from 19 patients). The primary outcome was wound area reduction, expressed as the wound healing rate; secondary outcomes included pain intensity and infection prevention.

RESULTS: The blue AgNPs and reference hydrogels were comparable in terms of wound area reduction and pain scores during the changing of wound dressings, with no significant differences ($p > 0.05$). Patients in the blue AgNPs hydrogel group showed low rates of bacterial infection for both gram-negative and gram-positive strains; in particular, there was almost complete prevention of infection by gram-positive strains at day 21 after treatment initiation.

CONCLUSION: The blue AgNPs hydrogel may be effective in preventing bacterial infections of both gram-negative and gram-positive strains at 14–21 days. Thus, the blue AgNPs hydrogel is a promising material for therapeutic applications in wound care.

KEYWORDS: antimicrobial agents, hydrogel, nanoparticles, wound dressing

INTRODUCTION

Wound healing, as a protective function of the skin upon injury, is a complex process, whereby damaged tissue is restored by the formation of connective tissue and regrowth of the epithelium[1]. For acute wounds, the wound healing process is completed in a timely fashion, whereas chronic wounds are those that fail to heal within 4 weeks[2].

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A wound dressing can be directly applied to the wound, enhancing the healing process. Various types of wound dressing materials, such as films, foams, hydrogels, and hydrocolloids, have been employed for the treatment of skin ulcers. In the management of acute and chronic wounds, a range of materials can be utilized to stimulate wound healing and establish an optimal environment for tissue regeneration. Commonly used materials for acute and chronic wounds include traditional dressings, hydrocolloids, hydrogels, foams, alginates, antimicrobial dressings, and negative pressure wound therapy[3]. Hydrogels are advanced wound dressings composed of water-insoluble hydrophilic materials, which are typically synthetic or composite polymers. Their soft and elastic nature facilitates easy application and promotes epithelium progression by maintaining a moist environment. Hydrogels are an optimal choice for wound dressings as they effectively create a moist environment at the wound site, assist in the removal of wound exudates, prevent infection, and establish a suitable environment for tissue regeneration[4-6]. According to Dumville et al., hydrogel dressings have demonstrated promising potential in accelerating the healing process of lower grade diabetic foot ulcers when compared to basic wound contact dressings[7]. Shu et al. highlighted three key advantages of using hydrogel dressings for burn wound treatment. Firstly, hydrogel dressings possess excellent absorbent properties, capable of absorbing a significant amount of wound exudate relative to their dry weight. This feature helps maintain a moist environment during wound healing, particularly beneficial for dry wounds. Secondly, the versatility of hydrogel dressings allows for customization to suit the specific shape and condition of the wound. Lastly, hydrogel dressings provide non-adhesive adherence to wounds, thereby reducing both temperature and pain. Additionally, their transparent nature allows for wound observation[8].

Silver nanoparticles (AgNPs) have attracted interest for use in clinical applications because of potential biological properties, including antibacterial activity and wound healing efficacy. When AgNPs are applied to a wound, they attach to and penetrate the cell membrane of bacteria and preferentially attack the respiratory chain and cell division, ultimately leading to cell death[9-10]. Several reports have demonstrated that the antibacterial potency of AgNPs is size-dependent[11-12]. Small AgNPs are more likely than large AgNPs to cross the cell membrane and enter the cell, increasing the toxicity to bacteria. Smaller AgNPs have a larger specific surface area; therefore they exert stronger antibacterial effects than larger AgNPs[13]. Although numerous studies on the efficacy of hydrogel with AgNPs have been published[14-16], information on a hydrogel with AgNPs produced and developed in a developing country is lacking.

Recently, a novel stable hydrogel that composed of a patented carbomer, blue AgNPs, glycerol, and water, was developed in Thailand. The blue AgNPs hydrogel is prepared by dispersing carbomer, bio-cellulose powder, and glycerol in distilled water and adding blue AgNPs to the dispersion at a concentration of 30 ppm. The carbomer polymer chosen for the hydrogel preparation is a self-wetting polymer, providing moderate to high viscosity as well as stabilizing and bioadhesive properties for hydrogel applications. The carbomer can also imbue amorphous hydrogels with moisturizing and absorption properties that facilitate the autolysis and softening of dead tissue. Thus, the blue AgNPs hydrogel was designed to optimize a moist wound environment, providing pain relief during wound care, and to prevent wound colonization by microorganisms.

Pain during the changing of the wound dressing is another important issue. Some patients undergo multiple painful changes during their wound care, which can cause unfavorable physiological and emotional effects. A hydrogel dressing can provide pain relief due to an evaporative cooling effect and create a moist environment, which soothes exposed nerve endings in the skin[17]. Moreover, silver dressings may relieve pain by providing a moist and protective air-free wound environment and can be left in place for some period of time[18].

This study was performed to investigate the efficacy of the blue AgNPs hydrogel compared to a reference hydrogel. The reference hydrogel was selected as the comparative group due to its shared substrate characteristics with advanced wound dressings, similar to the blue AgNPs hydrogel being evaluated. By comparing the efficacy of these hydrogels, the study sought to assess their relative performance. It is worth noting that other advanced wound dressings, such as hydrocolloid, hydrofiber, and foam, have been shown to potentially accelerate wound healing, but this particular study focused on comparing the substrate properties of the products. Additionally, the incorporation of blue AgNPs into the hydrogel serves the purpose of providing infection protection. Specifically, the aim of this study was to investigate the clinical efficacy of the blue AgNPs hydrogel in terms of wound healing, pain intensity, and antimicrobial prevention in acute and chronic wounds.

METHODS

This prospective study evaluated the effectiveness of the blue AgNPs hydrogel as a primary aid for acute and chronic wounds compared with that of a reference material. This study was conducted in accordance with the Helsinki Declaration of Human Rights and approved by the Research Ethics Committee of the Faculty of Medicine Vajira Hospital, Navamindradhiraj University, Thailand (study code 046/60). This study was also registered at Thai Clinical Trials Registry (TCTR), number TCTR20230623001.

Recruitment was conducted by attending physicians and nursing staff at the Outpatient Wound Clinic of the Department of Surgery at Vajira Hospital, Thailand, from September 2017 to September 2018. All participants were required to be >10 years of age and have acute or chronic partial- or full-thickness wounds; wounds due to thermal burns and accidental injury were included. Patients who were pregnant, allergic to hydrogel or silver, had a compromised immune system, or had any other connective tissue disease were excluded. Infected wounds with frank pus or necrotic tissues were also excluded.

Once written informed consent was obtained, wound sites were randomly stratified to receive the blue AgNPs hydrogel or the reference hydrogel (as control) using a computerized random number sequence-generating program. Both treatments were applied according to the manufacturer's instructions, followed by standard wound care, typically consisting of cleaning, debridement, and dressing application once daily. Patients underwent dressing changes and were followed up prospectively until full healing or 21 days (figure 1).

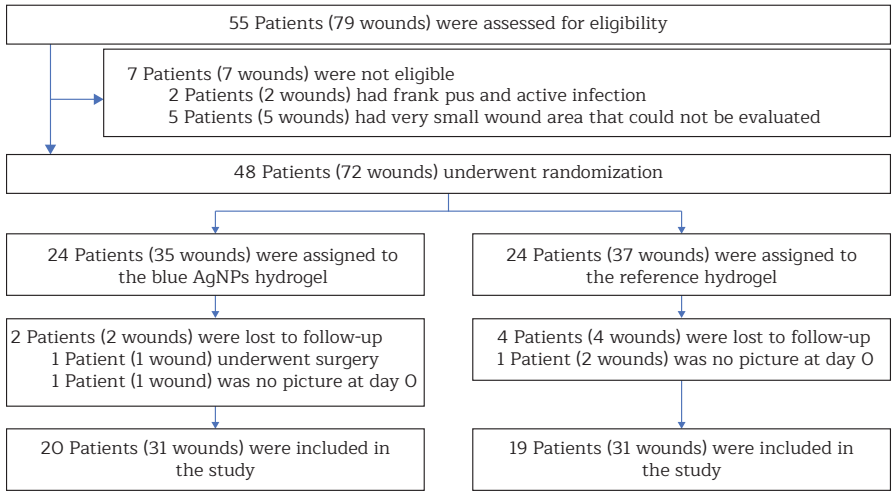

Figure 1 Flow diagram of a single trial of the blue AgNPs hydrogel and the reference hydrogel for acute and chronic wound management. The diagram illustrates a single-center trial with parallel randomized trial of two groups.

The blue AgNPs hydrogel (Novatec Healthcare, Samut Prakan, Thailand) was used as the experimental material. The dressing consisted of carboxymethyl cellulose polymer, blue AgNPs, glycerol, and water. The size- and shape-dependent antibacterial activities of the blue AgNPs hydrogel have been measured using optical density and fluorescence intensity, and their absorbency has been measured with an ultraviolet spectrophotometer. Additionally, characterization results obtained through transmission electron microscopy analysis have documented the different sizes (40–100 nm) and shapes (spherical, cuboid, and planar) of the blue AgNPs hydrogel[19]. The blue AgNPs hydrogel was sterilized by autoclaving in aluminum tubes. A commercially available carbomer-based hydrogel (IntraSite®; Smith & Nephew, Watford, UK) was used as the reference material. IntraSite® gel is composed of 3% carboxy-methylcellulose polymer, 77% water, and 20% propylene glycol. The water in the hydrogel only partially hydrates the polymer, allowing it to maintain its absorptive capacity. As a result, the gel effectively absorbs excess exudate from the wound site, reducing the likelihood of leakage[20].

The primary outcome was wound area reduction, expressed as the wound healing rate. The wound area was evaluated on days 0, 7, 14, and 21 after treatment initiation[21-22]. All assessors were blinded to treatment group allocation prior to measuring the wounds. The wound area was measured by three well-trained practitioners using a centimeter ruler and the scratch wound healing assay, which relies on ImageJ[23-24]. The mean wound area across three practitioners was used for calculation as the percentage of wound area reduction as follows:

$$\% \text{ wound area reduction} = \frac{Ao - At}{Ao} \times 100\%,$$

where *Ao* is the original wound area and *At* is the area of the wound at the time of the observation.

Secondary outcome measures included pain intensity during dressing changes and infection prevention. Pain intensity during dressing changes on days 0, 7, 14, and 21 was assessed using a 10-point visual analogue scale, whereby a score of 0 indicated no pain and a score of 10 indicated severe pain[18,25].

The microbiological flora was sampled using wound surface swabs obtained during dressing changes on days 0, 7, 14, and 21. Bacteria were identified and quantified using standard microbiological techniques. The wound swab was cultured to identify microbial infections using colonization, graded as 1+ (10^2–10^3 CFU/g), 2+ (10^3–10^4 CFU/g), or 3+ (10^5 CFU/g), according to the bacterial growth on a culture plate[26-27].

All values are expressed as mean ± standard deviation, and statistical analyses were performed using SPSS version 18.0 (IBM Corp., Armonk, NY, USA). Differences between treatment groups were evaluated using the Student t-test for data with normal distribution and the Mann-Whitney U test for data with non-normal distribution. p-values < 0.05 were considered statistically significant.

RESULTS

A total of 62 wound sites from 39 patients (age, 57 ± 19 years; range, 14–84 years) were enrolled. The majority of patients had thermal burns and accidental injuries. The baseline characteristics of the enrolled patients are presented in Table 1. In total, 31 wound sites from 20 patients were randomized to receive the blue AgNPs hydrogel and 31 wound sites from 19 patients were randomized to receive the reference hydrogel.

Table 1 Demographics and clinical baseline characteristics

Characteristics	Blue AgNPs hydrogel	Reference hydrogel	P-value
Patients, n	20	19	
Age, years	59 ± 3	54 ± 4	0.317
Sex, F/M	6/14	9/10	
Comorbidities			
Diabetes, n	12	8	
Hypertension, n	9	5	
DLP, n	2	1	
CAD, n	2	2	
Etiology (sites), n (area; cm^2)	31 (8.3±2.3)	31 (4.9±1.1)	0.197
Acute wound (sites), n (area; cm^2)	24 (8.3±2.8)	23 (5.4±1.4)	0.361
Flame burn (sites), n	12	10	
Accident (sites), n	10	10	
Abrasion (sites), n	2	3	
Chronic wound (sites), n (area; cm^2)	7 (8.4±3.7)	8 (3.6±1.1)	0.202
Location (sites), n			
Scalp	2	–	
Neck		1	
Chest	–	2	
Back	1	1	
Leg	11	14	
Forearm	1	6	
Buttock	3	–	
Foot	8	6	
Hand	5	1	

Abbreviations: AgNPs, silver nanoparticles; CAD, coronary artery disease; cm^2, square centimeters; DLP, dyslipidemia; F, female; M, male; n, number of patients

Overall, the two groups showed no significant differences in wound area reduction or residual wound healing rate. Both treatments showed reduced wound area in a time-dependent manner (figure 2). In the blue AgNPs hydrogel group, the area of acute wounds decreased from 8.3 ± 2.8 cm^2 on day 0 to 3.2 ± 2.2 cm^2 (61% wound area reduction) on day 7. This reduction continued gradually, reaching 0.7 ± 1.5 cm^2 (91% wound area reduction) by day 21. Conversely, in comparison, chronic wounds exhibited delayed wound closure. The area of chronic wounds decreased from 8.4 ± 3.7 cm^2 on day 0 to 5.9 ± 3.2 cm^2 on day 7, indicating partial wound closure (30% wound area reduction). Nevertheless, by day 21, significant healing was observed in chronic wounds, with a wound area reduction of 75%. In the reference hydrogel group, the area of acute wounds decreased from 5.4 ± 1.4 cm^2 on day 0 to 2.2 ± 0.8 cm^2 on day 7. This reduction continued gradually, reaching 0.3 ± 0.1 cm^2 (94% wound area reduction) by day 21. Regarding chronic wounds in this group, the area decreased from 3.6 ± 1.1 cm^2 on day 0 to 2.0 ± 0.63 cm^2 on day 7, indicating partial wound closure (42% wound area reduction). By day 21, substantial healing was observed in chronic wounds, with a wound area reduction of 83% (figure 3A).

There was no significant difference in the reduction of pain intensity during dressing changes between the two treatment groups. Patients in both the blue AgNPs hydrogel group and the reference hydrogel group exhibited a consistent decrease in pain scores over time for both acute and chronic wounds. In the acute wound subgroup treated with the blue AgNPs hydrogel, the mean pain score at day 14 was minimal (range, 0.25–0.71), and by day 21, pain during dressing changes had completely subsided. Similarly, in the reference hydrogel group, the mean pain scores at day 14 and 21 were negligible (range, 0.05–0.29 and 0.04–0.22 respectively) (figure 3B). In the case of chronic wounds, both treatment groups displayed a declining trend in pain scores, without any significant differences.

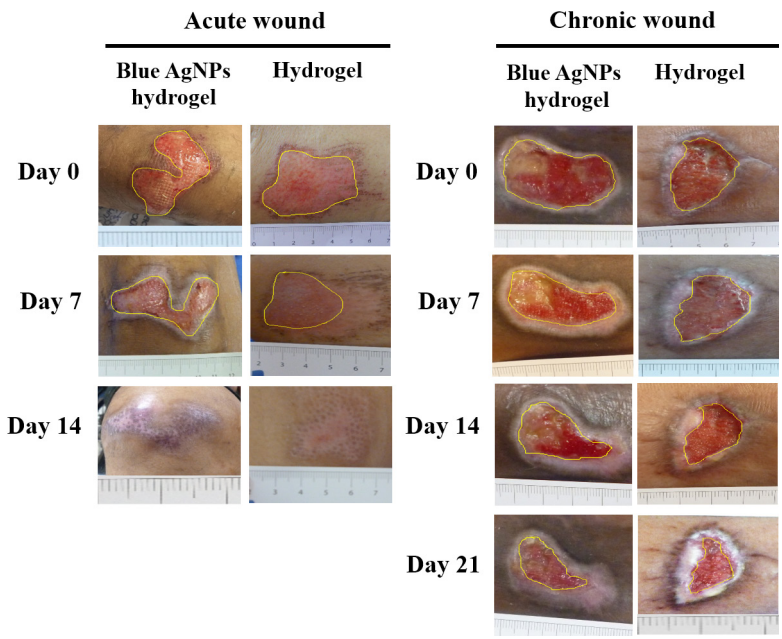

Figure 2 Representative photographs showing wound closure in acute and chronic wounds treated with the blue silver nanoparticles hydrogel or the reference hydrogel. The wound area, outlined in yellow, was assessed using ImageJ during dressing changes on days 0, 7, 14, and 21 after treatment initiation.

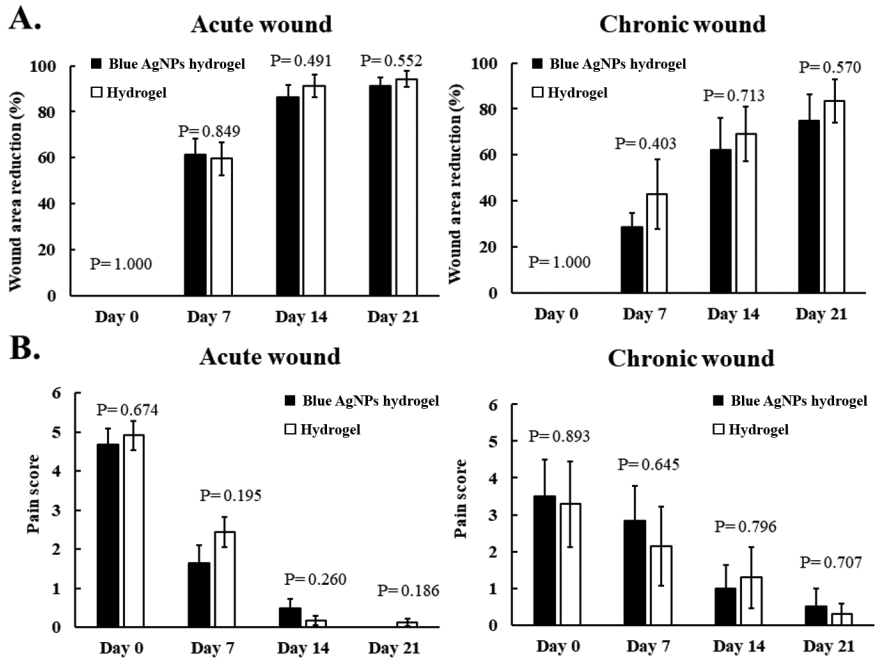

Figure 3 Wound area reduction and pain intensity during dressing changes on days 0, 7, 14, and 21 for acute and chronic wounds. (A) Both the blue silver nanoparticles hydrogel and reference hydrogel induced wound closure over time in acute (n = 24 and n = 23, respectively) and chronic wounds (n = 7 and n = 8, respectively), without significant group differences. (B) Both the blue silver nanoparticles hydrogel and reference hydrogel reduced pain intensity scores over time, with a greater decrease for acute wounds (n = 24 and n = 23, respectively) than for chronic wounds (n = 7 and n = 8, respectively) on days 14 and 21. All data are expressed as mean ± standard deviation.

Regarding infection prevention, the blue AgNPs hydrogel group demonstrated low rates of bacterial infection for both gram-negative and gram-positive strains, as shown in Table 2. In particular, gram-positive strains were more susceptible to prevention than gram-negative strains, with almost complete prevention at day 21 after treatment initiation. Importantly, no clinical signs of wound infection, such as the presence of frank pus or increased pain, were observed in either group. Furthermore, the wounds in both groups exhibited a gradual healing process.

DISCUSSION

This prospective study is the first to report the efficacy of a blue AgNPs hydrogel, produced and developed in a developing country, in wound healing and infection prevention in acute and chronic wounds. The study results show that the blue AgNPs hydrogel can induce complete wound healing in acute and chronic wounds in as short as 14 days. Generally, acute wounds tend to heal within 3 weeks, whereas chronic wounds tend to persist for a minimum of 3 months after the injury[28]. In the present study, 25 (80%) of 31 sites treated with the blue AgNPs hydrogel had completely healed by day 14. This result is similar to the re-epithelialization results observed with chitosan cross-linked materials, for which the rate of wound healing was approximately 82.5% at day 14[29]. The blue AgNPs hydrogel matrix has a cross-linked hydrophilic biopolymer and high water content, providing good conditions for maintaining a humid environment around the wound interface prior to immune cell activation and increasing the speed of wound healing[30-31]. In the present study, the observation period was 21 days because wound area reduction and complete wound healing could be observed within 14–21 days.

Additionally, the time for re-epithelialization using wound dressings with AgNPs has been widely studied. A previous multicenter study demonstrated a significantly faster healing time with a silver-containing soft silicone foam dressing than with standard care; the average time to complete healing was 13.44 days for 75% of patients treated with the foam[32]. Similar re-epithelialization results were shown for AgNPs embedded into a chitosan-polyethylene glycol (PEG) hydrogel, as a substantial layer of dermal skin and mixed pattern of collagen were detected in the AgNPs-impregnated chitosan-PEG hydrogel group at day 14[14].

Table 2 Comparison in bacterial colonization between different wound dressing biomaterials

Bacterial stain	Blue AgNPs hydrogel					Reference hydrogel				
	Wound site (n)	Day				Wound site (n)	Day			
		0	7	14	21		0	7	14	21
Gram-negative										
Acinetobacter baumannii	2	–	–	1+	1+	2	2+	–	–	1+
Escherichia coli	3	1+	–	–	1+	2	1+	1+	1+	–
Klebsiella pneumoniae	4	–	–	1+	1+	1	–	3+	1+	2+
Pseudomonas aeruginosa	1	–	1+	1+	–	1	1+	2+	1+	1+
Gram-positive										
Corynebacterium striatum	2	1+	–	–	1+	1	–	–	2+	1+
Enterobacter cloacae	1	–	1+	–	–	1	–	2+	2+	–
Staphylococcus aureus	3	1+	–	–		5	–	1+	–	1+
Staphylococcus caprae	2	–	1+	1+	–	1	–	–	1+	1+
Staphylococcus epidermidis	6	1+	1+	1+	–	3	1+	1+	–	–
Staphylococcus haemolyticus	7	1+	1+	–	–	2	1+	1+	–	–
Staphylococcus hominis	1	–	3+	–	–	1	–	–	3+	–
Staphylococcus warneri	1	–	1+	–	–	1	–	–	3+	–

Abbreviations: AgNPs, silver nanoparticles; CFU/g, colony-forming units per gram; n, number of wound sites
Microbial colonization was graded according to bacterial growth on a culture plate (–, no growth; 1+, 10^2–10^3 CFU/g; 2+, 10^3–10^4 CFU/g; 3+, 10^5 CFU/g).

As a wound management outcome, pain reduction plays a role in reducing patient anxiety and leads to improved compliance and participation in the treatment[33]. Dried-out dressings and aggressive adhesives are most likely to cause pain during dressing removal. Choosing the appropriate dressing can maintain the moisture balance, providing adequate moisture without causing maceration or desiccation, both of which impede healing[34]. In the present study, the mean pain intensity score during dressing change was not significantly different between the two groups. The pain scores were similar for acute and chronic wounds at late stages; however, a significantly faster reduction was noted for acute wounds.

Microbial infections caused by bacteria and fungi are a serious health problem, especially with respect to the wound-healing process, and can lead to tissue morbidity and sepsis, depending on the severity of the infection[35]. Hydrogels loaded with AgNPs offer a useful starting point in engineering wound dressing materials. AgNPs have potential against a broad range of bacteria and fungi because of their ability to generate reactive oxygen species and bind to bacterial cell membranes, thus leading to membrane damage[36-37]. *Staphylococcus aureus* and *Pseudomonas aeruginosa* are the most common bacteria isolated from chronic wounds[38]. The blue AgNPs hydrogel showed the maximum activity against resistant bacteria isolated from wounds, preventing bacterial infection and accelerating wound healing at day 21.

In summary, the blue AgNPs hydrogel and the reference gel demonstrated similar results in terms of wound healing and pain reduction. However, the blue AgNPs hydrogel demonstrated notable advantages over the reference hydrogel, specifically in its ability to effectively prevent bacterial infections, especially those caused by gram-positive strains. Furthermore, the blue AgNPs hydrogel exhibited no adverse clinical effects. These results indicate that the blue AgNPs hydrogel can play a role in infection prevention and has application prospects in wound care.

A limitation of this study is that it included only a small number of patients from a single center with a variety of wounds in the same or different patients. Additionally, the duration time for wound investigation was short (3 weeks). Moreover, we did not use biopsy for evaluations of wound infection or colonization. Thus, future studies that are multicenter in nature, with a longer examination period (for at least 8–12 weeks), with both treatment materials applied to the same wound (by dividing the wound into two parts), and with tissue biopsy are needed for more refined data.

CONCLUSION

Our findings conclude that the blue AgNPs hydrogel exhibits considerable promise as a therapeutic material in wound care applications. The results of this prospective study demonstrate the ability of the innovative blue AgNPs hydrogel to enhance wound healing, alleviate pain, and prevent infection. These positive outcomes underscore the promising utility of the innovative blue AgNPs hydrogel in clinical wound care. Based on these findings, we believe that the blue AgNPs hydrogel has potential for clinical utilization in the field of wound care. Further research and larger-scale studies should be conducted to substantiate these results and support the future widespread adoption of the blue AgNPs hydrogel in clinical practice.

CONFLICT OF INTEREST

The authors have no financial interest in any of the products or devices mentioned in this article.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to restrictions.

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