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Investigation of the antimicrobial properties of temperature-sensitive hydrogel containing silver sulfadiazine against various bacterial strains

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ABSTRACT

In burn wound management, infection poses a significant challenge, accounting for 75% of deaths in burn patients. Silver sulfadiazine is broadly used as an effective antibacterial agent for treating burns. Numerous researchers have explored various dosage forms of silver sulfadiazine, such as cream, ointment, topical spray, and hydrogel, for antimicrobial topical applications. Hydrogels offer appealing advantages over conventional drug delivery systems due to their sensitivity and responsiveness to stimuli, particularly temperature. Nevertheless, the comprehensive investigation of the antimicrobial properties of temperature-sensitive hydrogel containing silver sulfadiazine against different bacterial strains remains lacking. Thus, the main objective of the current study is to explore the antimicrobial properties of the temperature-sensitive hydrogel, incorporating silver sulfadiazine, against various bacterial strains colonized in burn wounds. To assess the antimicrobial activity of the temperature-sensitive hydrogel, inhibition zone diameters were measured against different types of Gram-positive strains (*Staphylococcus aureus, Enterococcus faecalis, Enterococcus faecium, and Streptococcus pyogenes*) and Gram-negative strains (*Escherichia coli and Klebsiella pneumoniae*). The synthesized silver sulfadiazine-loaded temperature-sensitive hydrogel exhibited remarkable antimicrobial efficacy against these bacteria. Notably, there was no significant difference in the inhibition zone diameter between the silver sulfadiazine-loaded temperature-sensitive hydrogel and the positive control ($p>0.05$). These findings affirm that the silver sulfadiazine-loaded temperature-sensitive hydrogel holds promise as a drug delivery medium, demonstrating excellent antimicrobial activity against various bacterial strains that colonized in burn wounds.

Keywords: *Temperature-sensitive hydrogel, Antimicrobial properties, Silver sulfadiazine, Bacterial strains*

1. INTRODUCTION

Dermatological conditions have emerged as one of the top 15 medical concerns that show an increase in healthcare expenditures in the past decade [1]. The efficacy of topical dermatological drug treatments significantly depends on the selection of an appropriate drug delivery medium. Advancements in life sciences and a growing market for dermatological products have led to the development of enhanced topical formulations and drug delivery systems [1]. Incidences of skin wounds rank among the most prevalent injuries affecting skin tissues, resulting from a variety of causes, including cuts and burns. Certain wounds present significant health risks, particularly chronic diabetic wounds and those associated with peripheral arterial diseases. Such severe wounds often exhibit delayed closure and require an extended healing period to facilitate proper recovery [2].

Colonization and infection present significant challenges in the management of burn wounds. Patients with burns are at greater risk of bacterial growth due to altered immune responses and compromised skin barrier function [3]. Burn injuries of all types compromise the skin's protective mechanisms, creating a favorable environment for the colonization of burn wounds by diverse microorganisms [4]. There are a number of ways to acquire these microorganisms, including skin contact, the respiratory system, and contact with environmental sources, such as healthcare personnel's hands [5]. In the initial stages, burn wounds are predominantly colonized by Gram-positive bacteria, including *staphylococci* and *enterococci*, including *Staphylococcus aureus* (*S. aureus*), which may lead to morbidity and mortality [6], [7], *Enterococcus faecalis* (*E. faecalis*) [8], *Enterococcus faecium* (*E. faecium*), and *Streptococcus pyogenes* (*S. pyogenes*) [9]. Gram-negative bacteria colonize the wound surface within a week of a

burn, including *Klebsiella pneumoniae* (*K. pneumoniae*) and *Escherichia* coli (*E. coli*) [4].

According to estimates, infections are responsible for 75% of deaths in burn patients [10]. Annually, over 180,000 individuals lose their lives to burn injuries in low- and middle-income nations [11]. Despite notable progress in wound care and treatment, the occurrence and fatality rates of burn-related infections have markedly increased in burn patients. For burn treatment, the widely utilized antibacterial agent is silver sulfadiazine, a combination of silver and sulfadiazine [7]. Silver sulfadiazine's antimicrobial effects result from its molecular breakdown into sulfadiazine and silver ions. The silver ions disrupt bacterial triphosphate synthesis, while sulfadiazine inhibits the synthesis of folic acid in bacteria [12]. Sulfadiazine's impact on inhibiting folic acid synthesis in bacteria ultimately leads to the inhibition of bacterial DNA replication, interfering with replication and transcription and resulting in bacterial cell death.

Even though silver sulfadiazine is considered an important antibacterial drug, the commercially available silver sulfadiazine forms, such as 1% w/w cream and ointment (as indicated in Table 1), require multiple applications, hindering the healing process and exposing patients to infectious agents [13]. Moreover, these non-biodegradable dosage forms lead to pain and trauma during removal before reapplication. To address these challenges, researchers have explored diverse silver sulfadiazine-containing dosage forms for antimicrobial topical applications, including topical spray, sponge, and hydrogel (as shown in Table 1). Among these, hydrogels hold significant promise due to their sensitivity and responsiveness to stimuli, particularly temperature. Recent advancements have also explored the synthesis of hydrogels using cellulose derived from lignocellulosic materials, such as agricultural residues (e.g., rice husk [14], oil palm empty fruit bunches [15]) and fruit waste (e.g., banana fiber waste [16]). These materials offer a biodegradable and renewable alternative for hydrogel formulation, improving biocompatibility and environmental sustainability [17]. Despite these advancements, a comprehensive investigation of the antimicrobial properties of temperature-sensitive hydrogel containing silver sulfadiazine against various bacterial strains remains lacking. Thus, this study aims to investigate the antimicrobial properties of the temperature-sensitive hydrogel, incorporating silver sulfadiazine, against diverse bacterial strains colonized in burn wounds.

2. MATERIALS AND METHODS

2.1. Materials

Oil palm empty fruit bunches were obtained from the Tennamaram palm oil mill, Malaysia. Pluronic F-127 (PF-127) was purchased from Sigma–Aldrich, Germany. Bacteria strains: *S. aureus* (ATCC® 6538), *E. coli* (ATCC® 25922), *E. faecalis* (ATCC® 51575), *E. faecium* (ATCC® 6057), *S. pyogenes* (ATCC® 12344), and *K. pneumoniae* (NCTC 13438) were acquired from Microbiologics Inc., USA. Nutrient agar and nutrient broth were purchased from HiMedia Laboratories Pvt. Ltd., India.

2.2. Synthesis of Temperature-sensitive Hydrogel and Silver Sulfadiazine Drug Loading

Cellulose extraction from oil palm empty fruit bunches was performed following the procedure outlined in our prior research studies [15], [18]. The method used to dissolve PF-127 and synthesize the temperature-sensitive hydrogel was similar to the one detailed in our earlier work [19], [20]. The specific formulation for the temperature-sensitive hydrogel is presented in Table 2. For silver sulfadiazine loading, 100 mg of silver sulfadiazine was added into 10 g of temperature-sensitive hydrogel at low temperature (2–8 °C) in sol-phase. Subsequently, the temperature-sensitive hydrogel with silver sulfadiazine was gently stirred at 200 rpm for 1 hr under low temperature (2–8 °C) to achieve a uniform and homogeneous silver sulfadiazine-loaded temperature-sensitive hydrogel [21].

2.3. Antimicrobial Activity of Temperature-sensitive Hydrogel

The antimicrobial effectiveness of the temperature-sensitive hydrogel was evaluated by measuring the inhibition zone diameter against a variety of bacterial strains, comprising *S. aureus, E. coli, E. faecalis, E. faecium, S. pyogenes,* and *K. pneumoniae*. Initially, nutrient agar medium was poured into sterilized petri plates and allowed to solidify at room temperature. Subsequently, a sterile bent glass rod was used to evenly spread 100 µL of bacterial suspension (10^8 CFU/mL) onto the nutrient agar surface to ensure confluent growth. Carefully, wells with a diameter of 6 mm were created in the nutrient agar plates using a sterile tip. Following this, 50 µL of the temperature-sensitive

Table 1. Therapeutic dosage form of silver sulfadiazine drug

Formulation	Reference
Silver sulfadiazine-commercial cream (brand name: Silvadene®)	[22], [23]
Silver sulfadiazine-commercial ointment (brand name: Dermazine)	[13]
Silver sulfadiazine-loaded thermo-sensitive hydrogel	[7]
Silver sulfadiazine-loaded arabinoxylan ferulate hydrogel	[8]
Silver sulfadiazine-loaded polyvinyl alcohol hydrogel	[13], [24]
Silver sulfadiazine-Topical spray	[25]
Silver sulfadiazine-chitosan and gelatin hydrogel sponges	[26]

Table 2. Formula of temperature-sensitive hydrogel

Formula	PF-127 polymer (w/v%)	Cellulose fibers (w/v%)	DI water (w/v%)
HG1	20	0.0	80.0
HG2		1.0	79.0
HG3		2.0	78.0
HG4		3.0	77.0

hydrogel loaded with silver sulfadiazine was placed into each well. The agar plates were incubated at 37°C for 24 hrs. After the 24-hr incubation period, ImageJ software (NIH, USA) was used to measure the diameter of the inhibition zone, and 10 measurements were taken to minimize analytical error. As a positive control, 1% w/w dissolved silver sulfadiazine was utilized. To determine any statistical differences in the inhibition zone diameter among the various temperature-sensitive hydrogels, a student's t-test was conducted using OriginPro software (OriginLab Corporation, USA).

3. RESULTS AND DISCUSSION

3.1. Antimicrobial Study of Temperature-sensitive Hydrogel

The antimicrobial efficacy of the silver sulfadiazine-loaded temperature-sensitive hydrogels was visually confirmed through Figure 1, Figure 2, and Figure 3, which depict photographs of the inhibition zones and their respective diameters against (*S. aureus* and *E. coli*), (*E. faecalis* and *E. faecium*), and (*S. pyogenes* and *K. pneumoniae*) bacterial strains, respectively. The presence of clear zones of inhibition surrounding the silver sulfadiazine-loaded temperature-sensitive hydrogels provided evidence of their potent antimicrobial activity against the tested bacterial strains. These clear zones represent circular areas where bacterial colonies were unable to grow, indicating the inhibitory effect of the hydrogels on bacterial proliferation.

The effectiveness of the silver sulfadiazine-loaded temperature-sensitive hydrogels against various bacterial strains was evaluated by measuring the inhibition zone diameter. A larger inhibition zone diameter theoretically indicates better antimicrobial activity of the tested species, signifying its ability to inhibit bacterial growth [27]. Figures 1-3 illustrate the inhibition zones for the silver sulfadiazine-loaded temperature-sensitive hydrogels and the positive control against *S. aureus, E. coli, E. faecalis, E. faecium, S. pyogenes, and K. pneumoniae*. Notably, there were no significant differences in the inhibition zone diameter between the silver sulfadiazine-loaded temperature-sensitive hydrogels and the positive control. This observation was further supported by statistical analysis using the student's t-test, where the p-values for the silver sulfadiazine-loaded temperature-sensitive hydrogels and the positive control were both higher than 0.05 (as summarized in Tables A.1-A.6 in Appendix A). These findings confirm that the incorporation of silver sulfadiazine into the temperature-sensitive hydrogel does not affect its antibacterial activity.

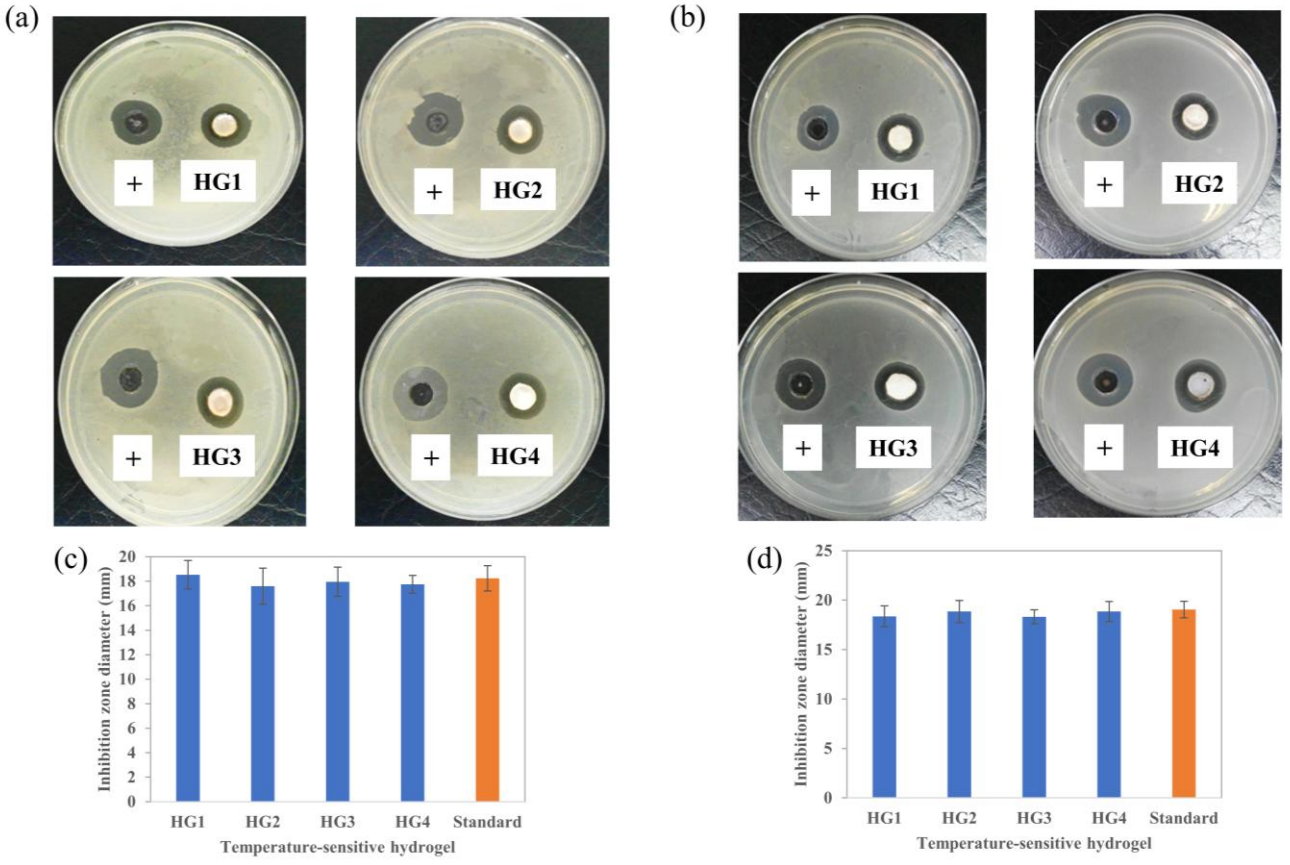

Figure 1. (a) and (b) Photographs of the inhibition zone on HG1-HG4 silver sulfadiazine-loaded temperature-sensitive hydrogels against *S. aureus* and *E. coli.* (c) and (d) Inhibition zone diameter of HG1-HG4 silver sulfadiazine-loaded temperature-sensitive hydrogels against *S. aureus* and *E. coli*

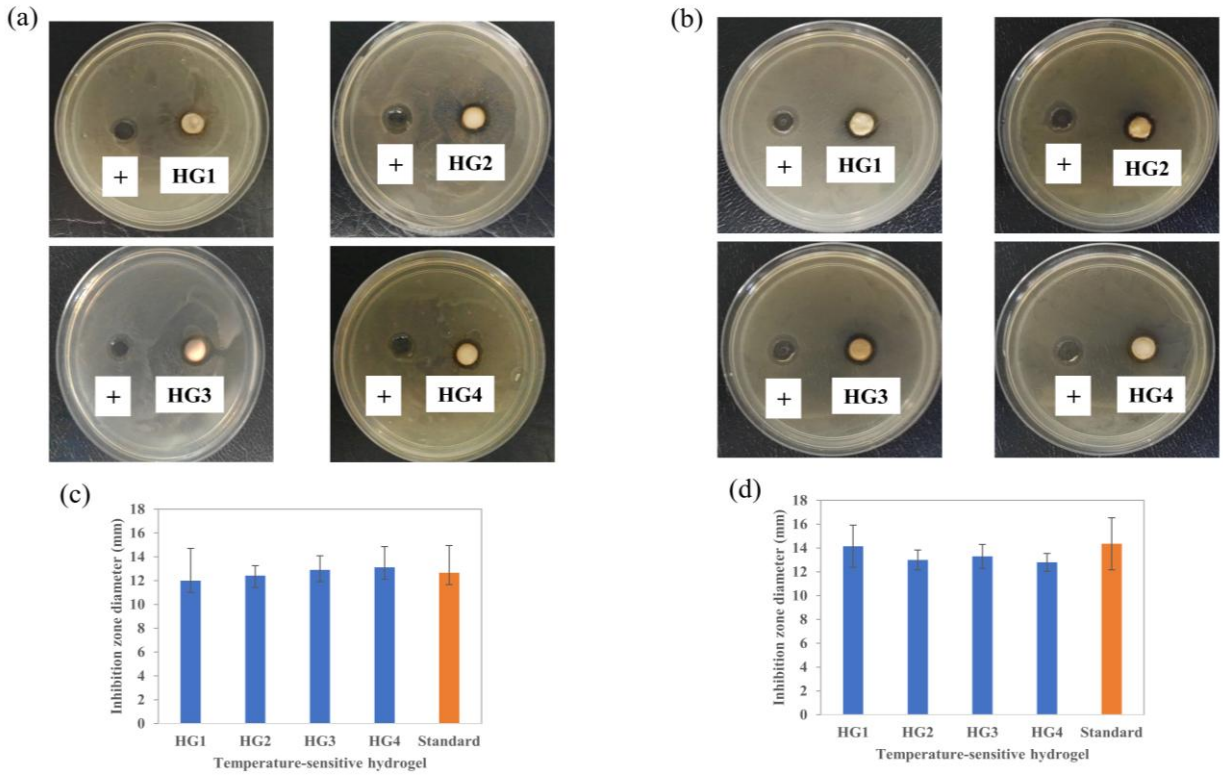

Figure 2. (a) and (b) Photographs of the inhibition zone on HG1-HG4 silver sulfadiazine-loaded temperature-sensitive hydrogels against *E. faecalis* and *E. faecium.* (c) and (d) Inhibition zone diameter of HG1-HG4 silver sulfadiazine-loaded temperature-sensitive hydrogels against *E. faecalis* and *E. faecium*

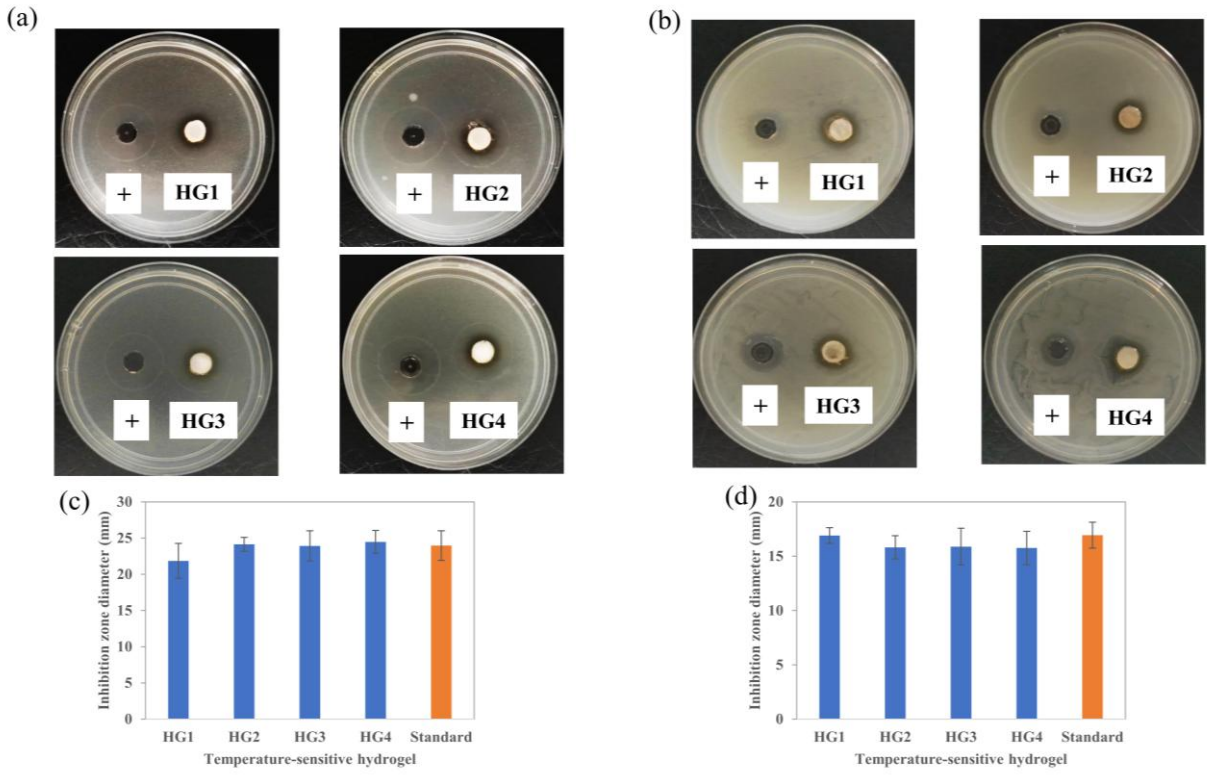

Figure 3. (a) and (b) Photographs of the inhibition zone on HG1-HG4 silver sulfadiazine-loaded temperature-sensitive hydrogels against *S. pyogenes* and *K. pneumoniae.* (c) and (d) Inhibition zone diameter of HG1-HG4 silver sulfadiazine-loaded temperature-sensitive hydrogels against *S. pyogenes* and *K. pneumoniae*

Comparison with the data in the literature, our previous study of 1% w/w silver sulfadiazine-loaded hydrogel, developed using a different formula, revealed that the silver sulfadiazine-loaded temperature-sensitive hydrogel in the current study exhibited almost similar inhibition zone diameters. Remarkably, the silver sulfadiazine-loaded temperature-sensitive hydrogel showed higher antibacterial effectiveness with larger inhibition zones compared to other 1 w/w% silver sulfadiazine/silver formulations, as presented in Table 3. Notably, when comparing its effectiveness against *S. pyogenes*, we referenced literature data on silver nanoparticles due to the absence of antimicrobial studies specifically on silver sulfadiazine against this bacterium. Specifically, our findings demonstrated larger inhibition zones against *S. aureus*, *E. coli*, and *K. pneumoniae* compared to a commercial cream, with inhibition zone diameters of 9.4 mm, 13.6 mm, and 11.7 mm, respectively. Furthermore, literature data on silver nanoparticles revealed even larger inhibition zones against *K. pneumoniae*, ranging from 15.0 mm to 22.0 mm, suggesting the potential superiority of silver sulfadiazine-loaded temperature-sensitive hydrogel over nanoparticle-based formulations.

Overall, the silver sulfadiazine-loaded temperature-sensitive hydrogel demonstrated higher efficacy against Gram-positive *S. pyogenes* bacteria, as evidenced by the larger inhibition zone diameter. This heightened efficacy against Gram-positive bacteria may be attributed to differences in cell wall composition between Gram-positive and Gram-negative bacteria, particularly the presence of peptidoglycan [9]. Gram-positive bacteria are characterized by a dense layer of peptidoglycan in their cell wall, as depicted in Figure 4. This structural feature enables the efficient absorption of drug molecules into the bacterial cell, owing to its porous nature. Consequently, this process effectively hinders bacterial reactivity [28]. In contrast, Gram-negative bacteria possess a thinner layer of peptidoglycan in their cell wall, accompanied by an outer membrane composed of lipopolysaccharides, lipoproteins, and phospholipids, which results in a complex cell wall structure. This complex cell structure, coupled with the presence of an outer membrane, acts as a barrier against the penetration of drug molecules [20]. Consequently, this structural complexity contributes to the reduced antimicrobial efficacy of the silver sulfadiazine-loaded temperature-sensitive hydrogel against Gram-negative bacteria.

Furthermore, the inhibition zone diameters of the silver sulfadiazine-loaded temperature-sensitive hydrogel varied across the tested bacterial strains, with the following order: *E. faecalis and E. faecium* (lowest) < *K. pneumoniae* < *S. aureus* < *E. coli* < *S. pyogenes* (highest). This trend could be linked to the minimum inhibitory concentration (MIC) of the silver sulfadiazine drug against each bacterium. The MIC values were determined as 50.0, 25.0, 100.0, 100.0, 12.5, and 50.0 mg/L for *S. aureus, E. coli, E. faecalis, E. faecium, S. pyogenes, and K. pneumoniae*, respectively [29]. A lower MIC value indicates higher antibacterial activity, requiring a smaller concentration of the silver sulfadiazine drug to inhibit bacterial growth [29]. Conversely, a higher MIC value necessitates a higher concentration of the drug to achieve effective inhibition. Hence, it is unsurprising that the silver sulfadiazine-loaded temperature-sensitive hydrogel showed the highest efficacy against *S. pyogenes*, which has the lowest MIC value for the silver sulfadiazine drug.

In comparison to our findings, a study by Ágnes Szegedi et al. (2014) [30] demonstrated that nanoporous silica particles loaded with silver sulfadiazine exhibited higher inhibition zone diameters against *S. pyogenes* bacteria compared to different types of bacteria such as *Pseudomonas aeruginosa*, *S. aureus,* and *E. coli*.

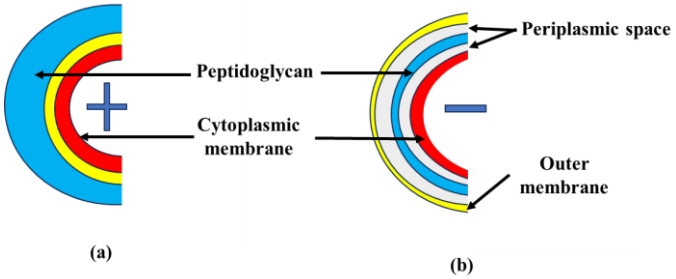

Figure 4. Illustration of cell wall structure of (a) Gram-positive bacteria and (b) Gram-negative bacteria

Table 3. Comparison of diameter of inhibition zone for various 1 w/w% silver sulfadiazine/silver formulations against different bacterial types

Name of bacteria / Type of bacteria	Diameter of inhibition zone (mm)				
	Temperature-sensitive hydrogel [This study]	**Hydrogel** [17]	**Commercial cream** [23]	**Commercial ointment** [13]	**Silver nanoparticles (12.5 mg/mL) [9]**
S. aureus / Gram-positive	17.6-18.2	17.8	12.6	9.4	--
E. coli / Gram-negative	18.3-19.1	19.6	13.6	--	--
E. faecalis / Gram-positive	12.0-13.1	13.7	--	--	--
E. faecium / Gram-positive	12.8-14.4	13.6	--	--	--
S. pyogenes / Gram-positive	21.9-24.5	23.2	--	--	15.0-22.0
K. pneumoniae / Gram-negative	15.7-16.9	16.5	11.7	--	--

The findings from this study provide confirmation that the antibacterial activity of the silver sulfadiazine drug doesn't change after its loading and release from the temperature-sensitive hydrogel. Moreover, the silver sulfadiazine-loaded temperature-sensitive hydrogel exhibits potential as a localized or topical treatment for diseases caused by the various bacterial strains evaluated in this research.

4. CONCLUSION

In conclusion, the silver sulfadiazine-loaded temperature-sensitive hydrogels demonstrated effective inhibitory activity against a variety of bacteria, including *S. aureus, E. coli, E. faecalis, E. faecium, S. pyogenes, and K. pneumoniae*. Notably, there were no significant differences in the inhibition zone diameter between the silver sulfadiazine-loaded temperature-sensitive hydrogels and the positive control. These findings highlight the promising antimicrobial potential of the silver sulfadiazine-loaded temperature-sensitive hydrogels, suggesting their viability as a therapeutic option for antimicrobial applications caused by the tested strains. The demonstrated efficacy, coupled with the absence of significant differences from the positive control, further confirms the practicality of integrating silver sulfadiazine into temperature-sensitive hydrogels for localized treatment. This successful integration of silver sulfadiazine with the hydrogel marks a significant enhancement of treatments of infectious diseases and wound management.

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APPENDIX A

Table A.1. p-value of inhibition zone diameter between HG1-HG4 temperature-sensitive hydrogel against *S. aureus*

Temperature-sensitive hydrogel	HG2	HG3	HG4	Positive control (standard)
HG1	0.10797	0.25843	0.07151	0.51222
HG2	--	0.54095	0.72578	0.25018
HG3	--	--	0.43155	0.55249
HG4	--	--	--	0.2084

Table A.2. P value of inhibition zone diameter between HG1-HG4 temperature-sensitive hydrogel against *E. coli*

Temperature-sensitive hydrogel	HG2	HG3	HG4	Positive control (standard)
HG1	0.78785	0.98566	0.32698	0.28948
HG2	--	0.7566	0.43072	0.09013
HG3	--	--	0.18602	0.05198
HG4	--	--	--	0.51281

Table A.3. p-value of inhibition zone diameter between HG1-HG4 temperature-sensitive hydrogel against *E. faecalis*

Temperature-sensitive hydrogel	HG2	HG3	HG4	Positive control (standard)
HG1	0.41814	0.16839	0.11269	0.5216
HG2	--	0.35505	0.08391	0.70067
HG3	--	--	0.64612	0.73035
HG4	--	--	--	0.6202

Table A.4. p-value of inhibition zone diameter between HG1-HG4 temperature-sensitive hydrogel against *E. faecium*

Temperature-sensitive hydrogel	HG2	HG3	HG4	Positive control (standard)
HG1	0.17683	0.67491	0.26081	0.77552
HG2	--	0.4308	0.58012	0.15517
HG3	--	--	0.2231	0.54036
HG4	--	--	--	0.21624

Table A.5. p-value of inhibition zone diameter between HG1-HG4 temperature-sensitive hydrogel against *S. pyogenes*

Temperature-sensitive hydrogel	HG2	HG3	HG4	Positive control (standard)
HG1	0.17742	0.12628	0.05395	0.09424
HG2	--	0.8366	0.64739	0.70292
HG3	--	--	0.51469	0.88155
HG4	--	--	--	0.75862

Table A.6. p-value of inhibition zone diameter between HG1-HG4 temperature-sensitive hydrogel against *K. pneumoniae*

Temperature-sensitive hydrogel	HG2	HG3	HG4	Positive control (standard)
HG1	0.07420	0.21616	0.08748	0.93657
HG2	--	0.59638	0.91577	0.12880
HG3	--	--	0.85524	0.16385
HG4	--	--	--	0.07270