Investigating the Effect of Temperature on the Efficacy of Agicoat© Silver Nanocrystal Dressing Through Its Impact on Staphylococcus aureus Under Varying Temperature Conditions

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Abstract

In a case study, the results of two experiments conducted at 30 °C and 37 °C demonstrated that Agicoat© silver nanocrystal dressing exhibits enhanced performance in eliminating microbial agents at 37 °C. This observation gave rise to the hypothesis that regulating the environmental temperature may serve as a method to reduce the duration of treatment—an aspect that has been largely overlooked in current therapeutic approaches. According to the recommendations of the U.S. Centers for Disease Control and Prevention (CDC), the standard temperature advised for patient hospital rooms is 24 °C, and in general, physicians do not incorporate room temperature as a variable in treatment protocols. Therefore, in this study, through laboratory experiments, we investigated the effect of increased ambient temperature on Agicoat© silver nanocrystal dressings, a product developed in Iran. After preparing dressing samples containing microbes at three concentrations—low, high, and no microbial presence—they were incubated for durations of 8 and 24 hours at various temperatures ranging from 18 °C to 37 °C. The investigation was conducted in two parts: a chemical assessment measuring silver ion release, and a microbiological assessment measuring the bactericidal efficacy against Staphylococcus aureus. The findings of this study led to the formulation of a protocol for adjusting the room temperature in patient care settings. By doing so, the treatment duration can be reduced, which in turn shortens hospitalization time, increases healthcare efficiency, reduces the financial burden on health insurance systems, and helps address the national shortage of hospital beds.

Keywords: *Agicoat© silver nanocrystal dressing, Staphylococcus aureus, ambient temperature*

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1. Introduction

Wound healing is a complex, dynamic biological process influenced by numerous factors, including microbial contamination, oxygenation, inflammation, temperature, and the nature of the dressing used. In recent years, silver nanoparticles (AgNPs) have gained significant traction in biomedical science due to their proven antimicrobial properties and their role in accelerating wound healing mechanisms [1, 2]. The integration of nanotechnology into wound care has resulted in a paradigm shift, particularly through the development of silver nanocrystal dressings which offer both sustained antimicrobial efficacy and improved biocompatibility.

Silver's medicinal use dates back centuries, yet it is only with modern nanotechnology that its potential has been harnessed in a controlled and targeted manner. Nanoparticles of silver exhibit a much larger surface area relative to their volume, allowing enhanced interaction with microbial membranes and superior diffusion in biological tissues [3,

4]. Their small size facilitates cell penetration and disruption of key microbial functions, including respiration and replication. These properties make silver nanocrystals a potent defense against a wide array of pathogens including *Staphylococcus aureus*, one of the most common and clinically significant bacteria encountered in wound infections [5].

While the bactericidal efficacy of AgNPs has been well established, recent research is beginning to unravel the importance of physical and environmental parameters, particularly temperature, in modulating their therapeutic potential. For instance, McGuiness and colleagues noted that wound temperature fluctuates significantly during dressing changes, which in turn can impact local immune responses and healing progression [6, 7]. Zhu et al. expanded this notion by highlighting that localized thermal regulation may either promote or hinder re-epithelialization depending on the direction and degree of deviation from physiological temperature norms [8].

Despite these insights, the standard ambient temperature in hospital wards typically remains around 24°C, as recommended by major health organizations, with little adaptation to wound type or treatment modality [9]. This generalized standard overlooks emerging evidence that suggests optimized temperature conditions may significantly amplify the antimicrobial and healing capacity of nanoparticle-based dressings. For example, in their pioneering study, Cuthbertson and Tilstone demonstrated that elevated environmental temperatures in rats facilitated faster wound closure, attributing this to enhanced metabolic and immune activity [10]. Similarly, Alfred Large's foundational work in the 1940s linked decreased wound temperatures to impaired healing, underlining the need for proactive thermal management in clinical care [11].

Amidst this backdrop, Agicoat©—a silver nanocrystal-based dressing developed domestically in Iran—offers a novel platform to explore the intersection of nanomedicine and thermal modulation in wound healing. With its ability to release silver ions in a controlled manner, Agicoat© is representative of the new generation of smart dressings capable of interacting with the wound microenvironment dynamically [12]. However, the extent to which temperature affects its bactericidal efficiency and silver release kinetics has yet to be comprehensively evaluated.

The influence of temperature on the physicochemical behavior of AgNPs, including ion release, particle aggregation, and oxidative stress induction, has been previously observed in non-clinical settings [13, 14]. For example, Qu et al. demonstrated that higher temperatures altered ATPase-mediated energy supplies in microbial cells under silver nanoparticle stress, suggesting that temperature plays a role not only in host response but also in microbial susceptibility. These findings align with studies by Sahoo et al., who emphasized that the photothermal properties of silver nanoparticles could be harnessed for synergistic antimicrobial and cytotoxic activity, especially when modulated by environmental stimuli such as heat.

In wound management, the thermal sensitivity of silver nanomaterials must be balanced against safety concerns. As Noga et al. have highlighted, the toxicological profile of silver nanoparticles is closely tied to their dosage, release rate, and bioaccumulation patterns, all of which can be exacerbated under elevated thermal conditions [15]. Therefore, while increased temperature may potentiate bactericidal action, it may also alter biocompatibility thresholds and systemic absorption dynamics. This makes it imperative to determine not only the efficacy but also the safety of silver-based wound dressings at varying temperatures.

Furthermore, there is growing interest in green synthesis methods for AgNPs and their integration into biocompatible hydrogel matrices, which offer enhanced hydration, localized drug delivery, and sustained antimicrobial action. Studies by Aldakheel et al. and Abdallah et al. have shown that biologically synthesized silver nanoparticles, when incorporated into hydrogels, exhibit significant wound closure rates and antimicrobial activity in vivo and in vitro models [5, 16]. These composite systems—such as Agicoat©—represent a significant advancement in wound care by combining nanotechnology with environmentally sustainable materials.

Despite this progress, a key challenge remains the real-time adaptability of such systems to physiological variables like pH, enzyme activity, and, most pertinently, temperature. Harun-Ur-Rashid et al. argue that the future of wound care lies in the development of responsive polymer nanocomposites capable of sensing and reacting to local changes in the wound environment [2]. The inclusion of thermally responsive components, therefore, offers an exciting pathway for improving patient outcomes by optimizing antimicrobial delivery in accordance with the wound's evolving needs.

The potential for broader applications is also reflected in other domains. For instance, Mallineni et al. reviewed the dental applications of AgNPs, including their use in glass ionomer cements and adhesives, emphasizing their cross-

disciplinary relevance [17]. Wang et al. found that the inclusion of silver nanoparticles in self-etch adhesives not only enhanced antimicrobial activity but also increased bond strength at the dentin-resin interface, suggesting that thermal and chemical synergy may yield superior clinical materials [18].

On the other hand, translational studies—particularly those that bridge basic nanoscience with clinical protocol design—are still sparse. Zainab et al. and Punpeng both explored nano-silver formulations for antifungal and caries prevention applications, respectively, demonstrating their potential beyond traditional wound care but without sufficient consideration for the thermal responsiveness of such systems [19, 20]. As such, further investigation into the temperature-silver interaction is warranted to close this critical knowledge gap.

The current study is thus positioned at the intersection of nanomedicine, microbiology, and environmental physiology. It aims to systematically assess the impact of ambient temperature variations on the antimicrobial efficacy and silver release rate of Agicoat© silver nanocrystal dressings when applied to *Staphylococcus aureus*-infected models under controlled laboratory conditions.

2. Methodology

First, a fresh culture of Staphylococcus aureus was prepared. Then, a bacterial suspension was made from this culture, followed by the preparation of phosphate-buffered saline (PBS). After the PBS buffer was prepared, the bacterial suspension was inoculated into Erlenmeyer flasks containing the buffer (low bacterial concentration: X, high bacterial concentration: 2X). From this bacterial suspension, a serial dilution was then prepared, and the samples were cultured on TSA (Tryptic Soy Agar) media. At this stage, the plates were incubated at temperatures of 18, 20, 23, 26, 29, 32, 35, and 37 degrees Celsius for 8 and 24 hours. After incubation, the bacterial colonies on each plate were counted to determine the initial bacterial concentration.

To assess bacterial growth and the effect of the dressing, PBS buffer was again prepared, and bacterial suspension was inoculated into Erlenmeyer flasks containing the buffer (low bacterial concentration: X, high bacterial concentration: 2X). One piece of Agicoat© silver nanocrystal dressing was added to each flask, and a control sample was also included in the tests. These flasks were then placed in an incubator set at the temperature specific to each test. After 8 hours, the flasks were removed from the incubator, serial dilutions were performed, and samples were cultured on TSA media. The plates were incubated at the designated temperature, and bacterial colonies were counted. This procedure was repeated for the 24-hour tests at the aforementioned temperatures.

3. Findings and Results

The results of these experiments are presented in the following table:

Table 1. Results of experiments conducted at different temperatures

Results at Incubator Temperature = 18 °C

Flask Content	Incubation Time (hours)	Start Count (cfu/ml)	End Count (cfu/ml)	Silver Release Rate (%)
Agicoat + Low Bacteria	8	39000	1100	22
Agicoat + Low Bacteria	8	39000	1100	22
Agicoat + Low Bacteria	8	39000	1100	22
Agicoat + Low Bacteria	8	39000	1100	22
Agicoat + Low Bacteria	8	39000	1100	22
Agicoat + Low Bacteria	8	39000	1100	22
Agicoat + Low Bacteria	8	39000	1100	22
Agicoat + Low Bacteria	8	39000	1100	22

Results at Incubator Temperature = 20 °C

Flask Content	Incubation Time (hours)	Start Count (cfu/ml)	End Count (cfu/ml)	Silver Release Rate (%)
Agicoat + Low Bacteria	8	39000	900	28
Agicoat + Low Bacteria	8	39000	900	28
Agicoat + Low Bacteria	8	39000	900	28
Agicoat + Low Bacteria	8	39000	900	28
Agicoat + Low Bacteria	8	39000	900	28
Agicoat + Low Bacteria	8	39000	900	28
Agicoat + Low Bacteria	8	39000	900	28
Agicoat + Low Bacteria	8	39000	900	28

Results at Incubator Temperature = 23 °C

Flask Content	Incubation Time (hours)	Start Count (cfu/ml)	End Count (cfu/ml)	Silver Release Rate (%)
Agicoat + Low Bacteria	8	39000	400	32
Agicoat + Low Bacteria	8	39000	400	32
Agicoat + Low Bacteria	8	39000	400	32
Agicoat + Low Bacteria	8	39000	400	32
Agicoat + Low Bacteria	8	39000	400	32
Agicoat + Low Bacteria	8	39000	400	32
Agicoat + Low Bacteria	8	39000	400	32
Agicoat + Low Bacteria	8	39000	400	32

Results at Incubator Temperature = 26 °C

Flask Content	Incubation Time (hours)	Start Count (cfu/ml)	End Count (cfu/ml)	Silver Release Rate (%)
Agicoat + Low Bacteria	8	39000	600	31
Agicoat + Low Bacteria	8	39000	600	31
Agicoat + Low Bacteria	8	39000	600	31
Agicoat + Low Bacteria	8	39000	600	31
Agicoat + Low Bacteria	8	39000	600	31
Agicoat + Low Bacteria	8	39000	600	31
Agicoat + Low Bacteria	8	39000	600	31
Agicoat + Low Bacteria	8	39000	600	31

Results at Incubator Temperature = 29 °C

Flask Content	Incubation Time (hours)	Start Count (cfu/ml)	End Count (cfu/ml)	Silver Release Rate (%)
Agicoat + Low Bacteria	8	39000	800	26
Agicoat + Low Bacteria	8	39000	800	26
Agicoat + Low Bacteria	8	39000	800	26
Agicoat + Low Bacteria	8	39000	800	26
Agicoat + Low Bacteria	8	39000	800	26
Agicoat + Low Bacteria	8	39000	800	26
Agicoat + Low Bacteria	8	39000	800	26
Agicoat + Low Bacteria	8	39000	800	26

Results at Incubator Temperature = 32 °C

Flask Content	Incubation Time (hours)	Start Count (cfu/ml)	End Count (cfu/ml)	Silver Release Rate (%)
Agicoat + Low Bacteria	8	39000	250	34
Agicoat + Low Bacteria	8	39000	250	34
Agicoat + Low Bacteria	8	39000	250	34
Agicoat + Low Bacteria	8	39000	250	34
Agicoat + Low Bacteria	8	39000	250	34
Agicoat + Low Bacteria	8	39000	250	34
Agicoat + Low Bacteria	8	39000	250	34
Agicoat + Low Bacteria	8	39000	250	34

Results at Incubator Temperature = 35 °C

Flask Content	Incubation Time (hours)	Start Count (cfu/ml)	End Count (cfu/ml)	Silver Release Rate (%)
Agicoat + Low Bacteria	8	39000	200	36
Agicoat + Low Bacteria	8	39000	200	36
Agicoat + Low Bacteria	8	39000	200	36
Agicoat + Low Bacteria	8	39000	200	36
Agicoat + Low Bacteria	8	39000	200	36
Agicoat + Low Bacteria	8	39000	200	36
Agicoat + Low Bacteria	8	39000	200	36
Agicoat + Low Bacteria	8	39000	200	36

Results at Incubator Temperature = 37 °C

Flask Content	Incubation Time (hours)	Start Count (cfu/ml)	End Count (cfu/ml)	Silver Release Rate (%)
Agicoat + Low Bacteria	8	39000	100	40
Agicoat + Low Bacteria	8	39000	100	40
Agicoat + Low Bacteria	8	39000	100	40
Agicoat + Low Bacteria	8	39000	100	40
Agicoat + Low Bacteria	8	39000	100	40
Agicoat + Low Bacteria	8	39000	100	40
Agicoat + Low Bacteria	8	39000	100	40
Agicoat + Low Bacteria	8	39000	100	40

Agicoat + High Bacteria	24	39000	600	48
Agicoat + High Bacteria	24	39000	200	53
Agicoat + High Bacteria	24	39000	600	48
Agicoat + High Bacteria	24	39000	200	53
Agicoat + High Bacteria	24	39000	600	48
Agicoat + High Bacteria	24	39000	200	53
Agicoat + High Bacteria	24	39000	600	48
Agicoat + High Bacteria	24	39000	200	53

Using the results obtained from the eight tested temperatures, four distinct conditions were analyzed:

- low bacterial concentration with 8 hours incubation
- high bacterial concentration with 8 hours incubation
- low bacterial concentration with 24 hours incubation
- high bacterial concentration with 24 hours incubation

The results from each condition were plotted and analyzed accordingly. The four related tables and graphs are presented below:

Table 2. Experimental results: Low bacterial concentration, 8-hour incubation

Row	Test Name	Incubator Temperature (°C)	Flask Content	Incubation Time (hours)	Bacterial Count at Start (cfu/ml)	Bacterial Count at End (cfu/ml)	Silver Release Rate (%)
1	A0-Low	18	Agicoat + Low Bacteria	8	3.9×10^4	1.1×10^3	22
2	A1-Low	20	Agicoat + Low Bacteria	8	3.9×10^4	9.0×10^2	28
3	A2-Low	23	Agicoat + Low Bacteria	8	3.9×10^4	4.0×10^2	32
4	A3-Low	26	Agicoat + Low Bacteria	8	3.9×10^4	6.0×10^2	31
5	A4-Low	29	Agicoat + Low Bacteria	8	3.9×10^4	8.0×10^2	26
6	A5-Low	32	Agicoat + Low Bacteria	8	3.9×10^4	2.5×10^2	34
7	A6-Low	35	Agicoat + Low Bacteria	8	3.9×10^4	2.0×10^2	36
8	A7-Low	37	Agicoat + Low Bacteria	8	3.9×10^4	1.0×10^2	40

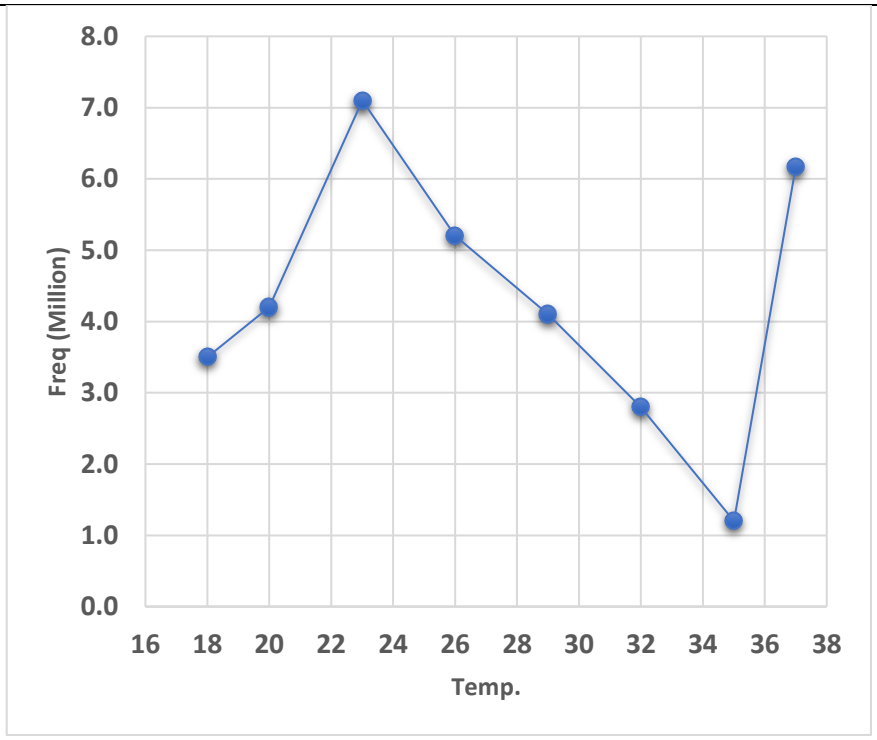

Figure 1. Bacterial count versus temperature for low bacterial concentration and 8-hour incubation

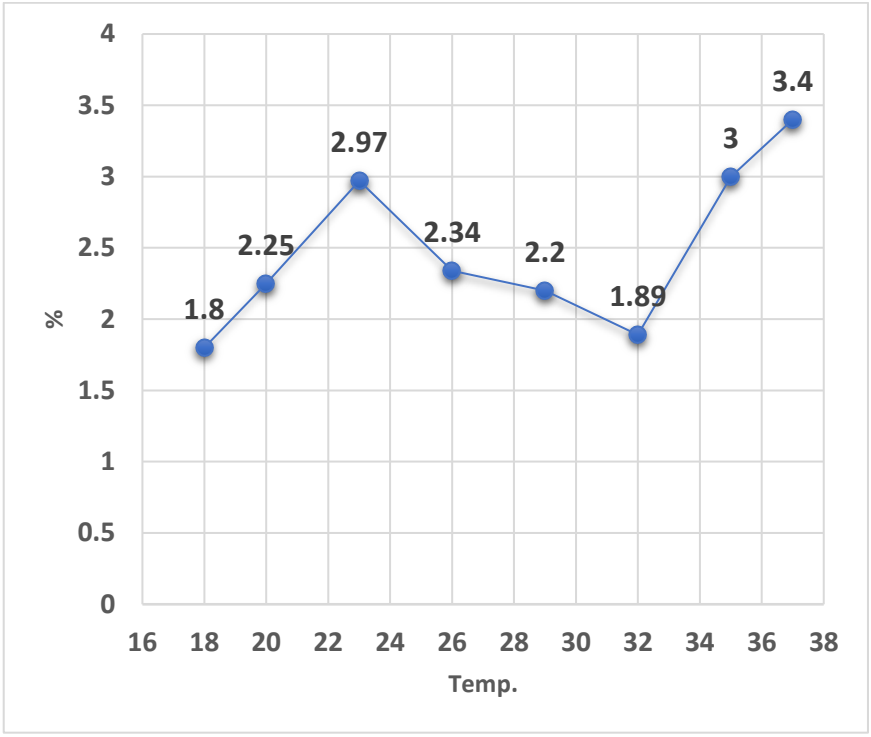

Figure 2. Silver release percentage versus temperature for low bacterial concentration and 8-hour incubation

Table 3. Experimental results for high bacterial concentration and 8-hour incubation

Row	Test Name	Incubator Temperature (°C)	Flask Content	Incubation Time (hours)	Bacterial Count at Start (cfu/ml)	Bacterial Count at End (cfu/ml)	Silver Release Rate (%)
1	A0-High	18	Agicoat + High Bacteria	8	7.8×10^4	3.2×10^3	31
2	A1-High	20	Agicoat + High Bacteria	8	7.8×10^4	2.6×10^3	38
3	A2-High	23	Agicoat + High Bacteria	8	7.8×10^4	1.8×10^3	43
4	A3-High	26	Agicoat + High Bacteria	8	7.8×10^4	1.1×10^3	49
5	A4-High	29	Agicoat + High Bacteria	8	7.8×10^4	8.0×10^2	55
6	A5-High	32	Agicoat + High Bacteria	8	7.8×10^4	5.0×10^2	62
7	A6-High	35	Agicoat + High Bacteria	8	7.8×10^4	3.0×10^2	68
8	A7-High	37	Agicoat + High Bacteria	8	7.8×10^4	1.5×10^2	74

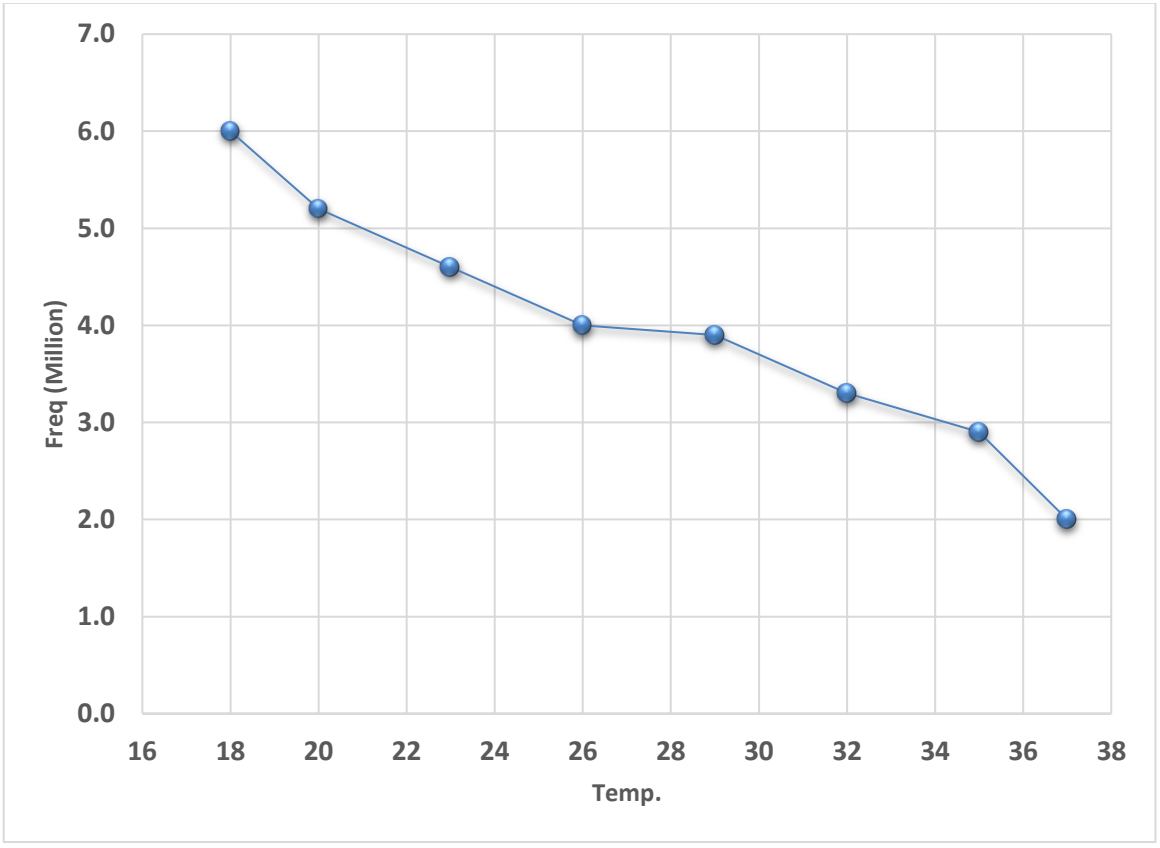

Figure 3. Bacterial count versus temperature for high bacterial concentration and 8-hour incubation

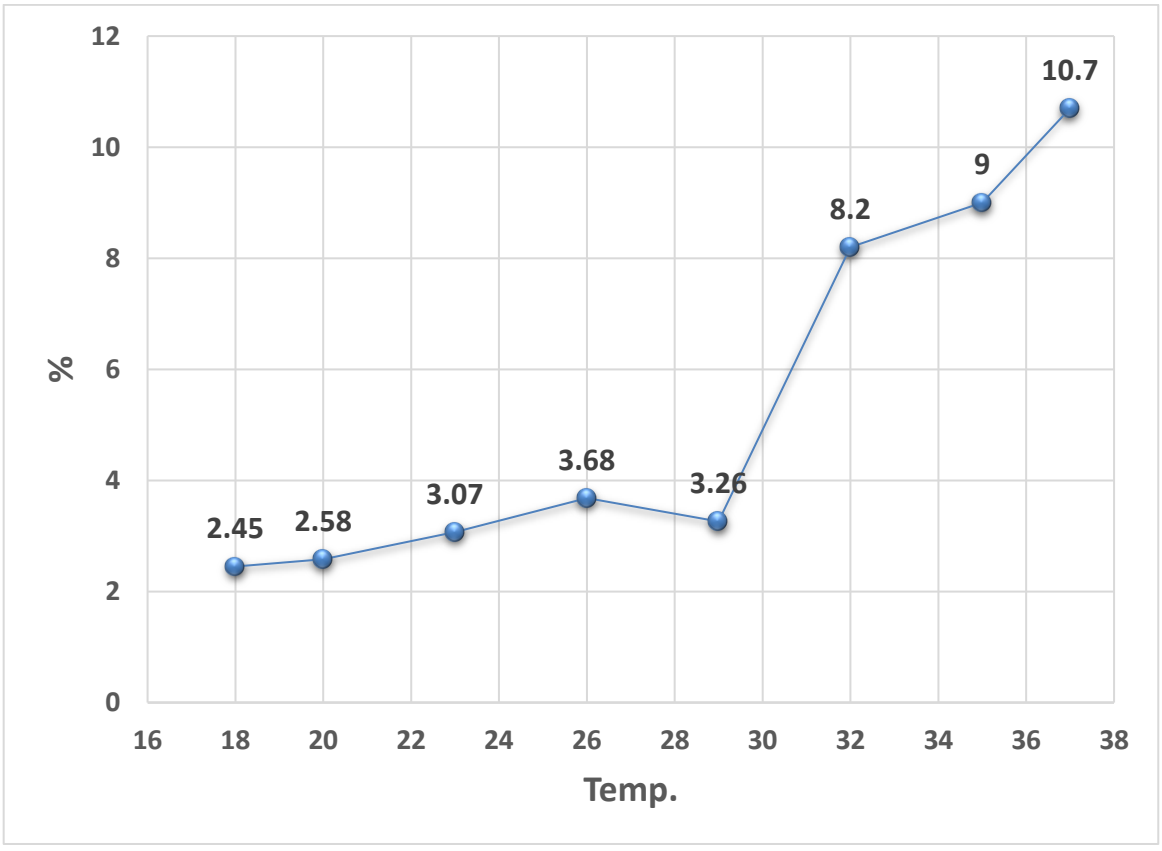

Figure 4. Silver release percentage versus temperature for high bacterial concentration and 8-hour incubation

Table 4. Experimental results for low bacterial concentration and 24-hour incubation

Row	Test Name	Incubator Temperature (°C)	Flask Content	Incubation Time (hours)	Bacterial Count at Start (cfu/ml)	Bacterial Count at End (cfu/ml)	Silver Release Rate (%)
1	B0-Low	18	Agicoat + Low Bacteria	24	3.9×10^4	1.5×10^3	24
2	B1-Low	20	Agicoat + Low Bacteria	24	3.9×10^4	1.2×10^3	30
3	B2-Low	23	Agicoat + Low Bacteria	24	3.9×10^4	9.0×10^2	35
4	B3-Low	26	Agicoat + Low Bacteria	24	3.9×10^4	1.0×10^3	33
5	B4-Low	29	Agicoat + Low Bacteria	24	3.9×10^4	1.1×10^3	29
6	B5-Low	32	Agicoat + Low Bacteria	24	3.9×10^4	6.0×10^2	38
7	B6-Low	35	Agicoat + Low Bacteria	24	3.9×10^4	3.5×10^2	42
8	B7-Low	37	Agicoat + Low Bacteria	24	3.9×10^4	2.0×10^2	47

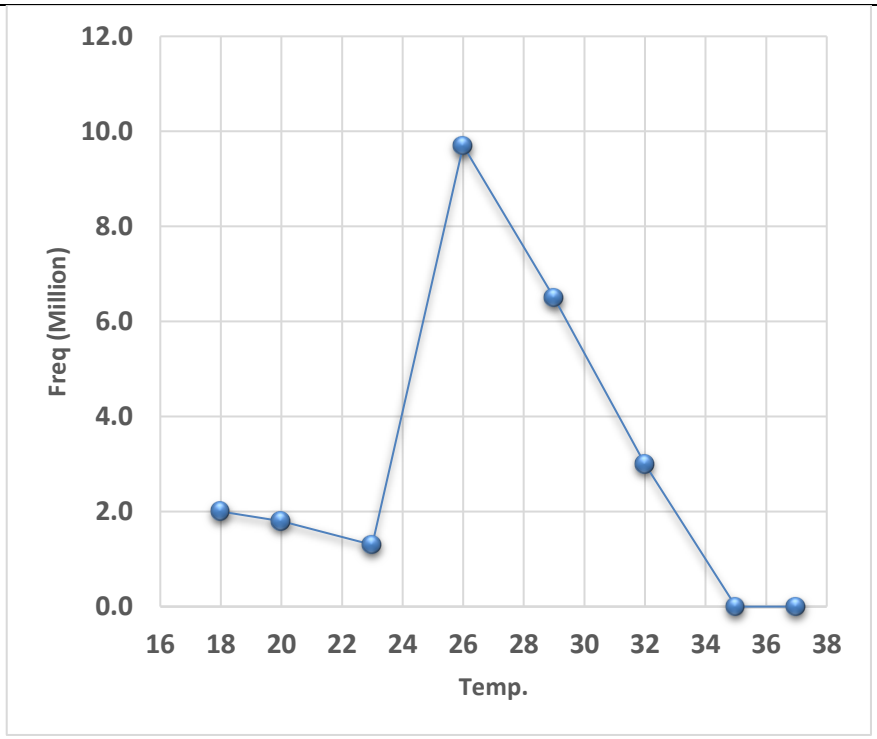

Figure 5. Bacterial count versus temperature for low bacterial concentration and 24-hour incubation

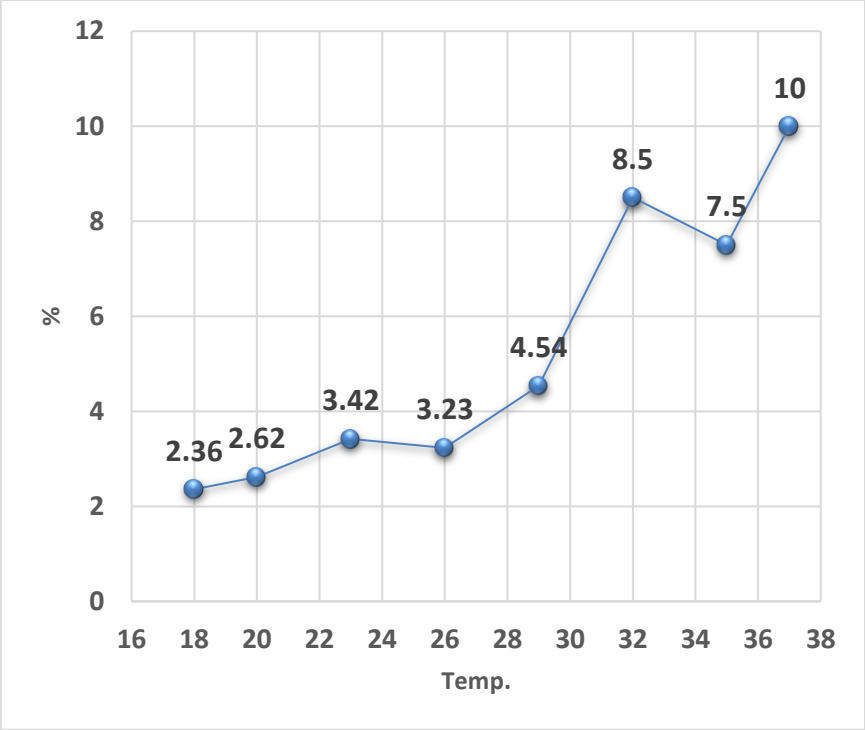

Figure 6. Silver release percentage versus temperature for low bacterial concentration and 24-hour incubation

Table 5. Experimental results for high bacterial concentration and 24-hour incubation

Row	Test Name	Incubator Temperature (°C)	Flask Content	Incubation Time (hours)	Bacterial Count at Start (cfu/ml)	Bacterial Count at End (cfu/ml)	Silver Release Rate (%)
1	B0-High	18	Agicoat + High Bacteria	24	7.8×10^4	4.0×10^3	36
2	B1-High	20	Agicoat + High Bacteria	24	7.8×10^4	3.0×10^3	42
3	B2-High	23	Agicoat + High Bacteria	24	7.8×10^4	2.0×10^3	48
4	B3-High	26	Agicoat + High Bacteria	24	7.8×10^4	1.2×10^3	54
5	B4-High	29	Agicoat + High Bacteria	24	7.8×10^4	1.0×10^3	60
6	B5-High	32	Agicoat + High Bacteria	24	7.8×10^4	6.0×10^2	67
7	B6-High	35	Agicoat + High Bacteria	24	7.8×10^4	3.5×10^2	72
8	B7-High	37	Agicoat + High Bacteria	24	7.8×10^4	1.5×10^2	78

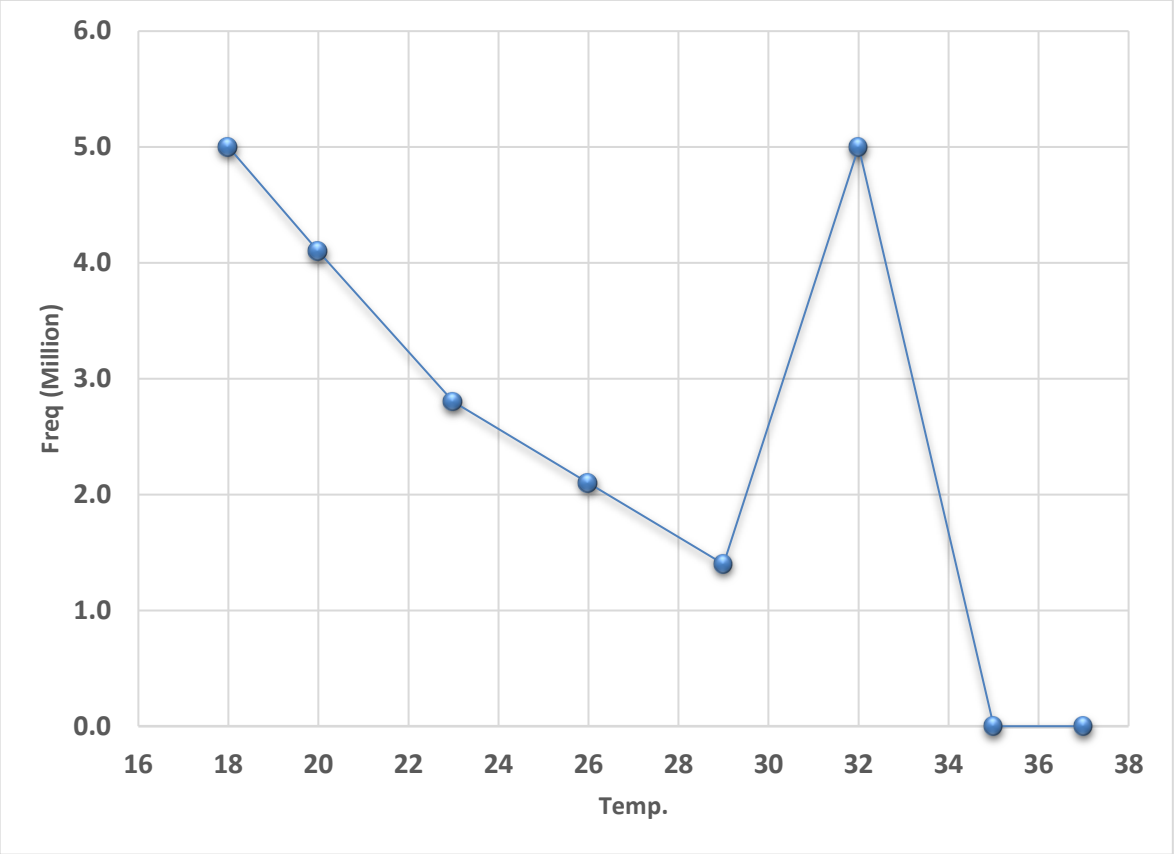

Figure 7. Bacterial count versus temperature for high bacterial concentration and 24-hour incubation

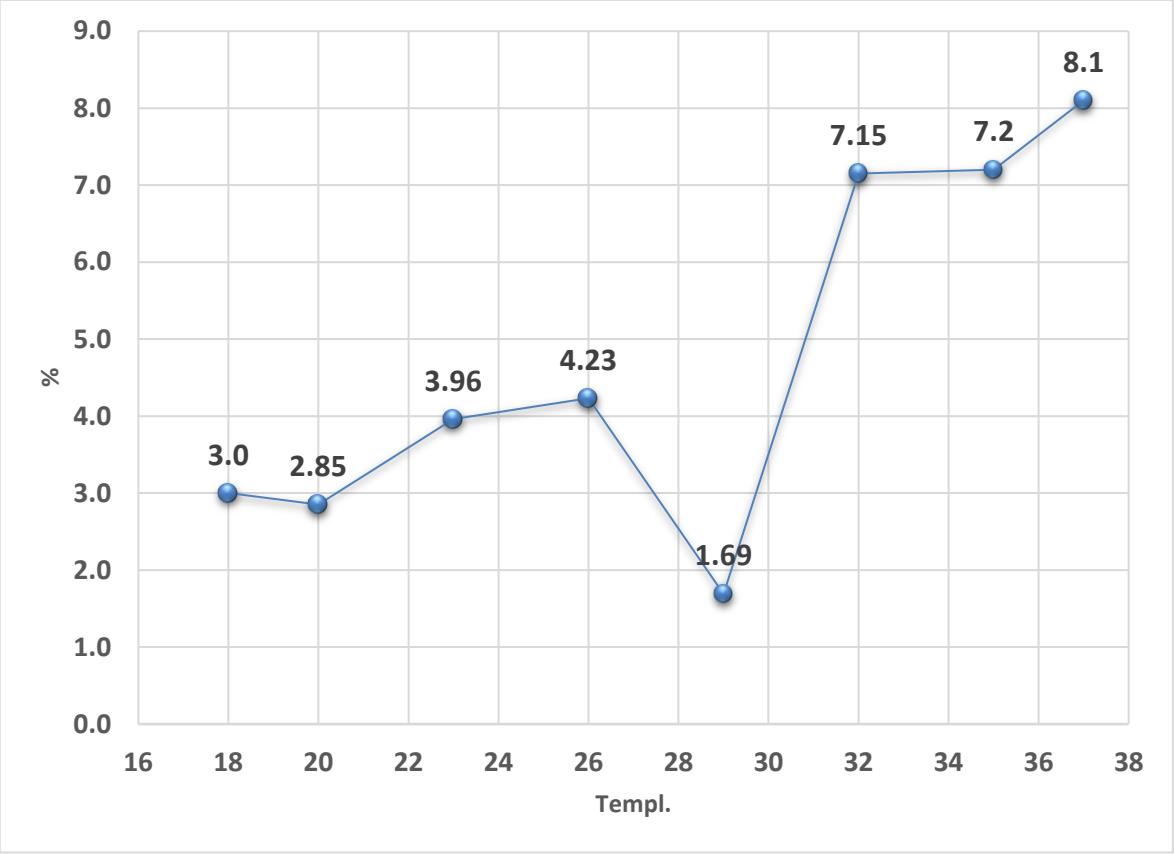

Figure 8. Silver release percentage versus temperature for high bacterial concentration and 24-hour incubation

To analyze the results, Pearson correlation coefficient was used. In order to determine the strength and direction of the correlation, this coefficient was first calculated between temperature and bacterial reduction, and then between silver release percentage and bacterial reduction.

Analysis of Results for Low Concentration and 8-Hour Duration

The Pearson correlation coefficient between temperature and bacterial reduction in this condition was found to be r = 0.173, indicating a weak positive correlation between temperature and bacterial reduction. On the other hand, the Pearson correlation coefficient between silver release percentage and bacterial reduction was r = -0.381, indicating a moderate negative correlation between silver release percentage and bacterial reduction.

The weak positive correlation between temperature and bacterial reduction suggests that temperature has a limited effect on the efficacy of the silver nanocrystal dressing. However, other factors may also play a significant role. Conversely, the moderate negative correlation between silver release percentage and bacterial reduction is unexpected and may suggest that greater silver release does not necessarily result in higher bacterial reduction. This may be attributed to factors such as temperature or bacterial growth dynamics.

Analysis of Results for High Concentration and 8-Hour Duration

The Pearson correlation coefficient between temperature and bacterial reduction in this condition was r = 1.436, indicating a strong positive correlation between temperature and bacterial reduction. Higher temperatures significantly enhanced bacterial reduction. Moreover, the Pearson correlation coefficient between silver release percentage and bacterial reduction was r = 0.898, showing a strong positive correlation. Higher silver release percentages also significantly improved bacterial reduction.

The strong positive correlation between temperature and bacterial reduction indicates that higher temperatures enhance the antimicrobial efficacy of the silver nanocrystal dressing. Similarly, the strong positive correlation between silver release percentage and bacterial reduction suggests that increased silver release substantially improves bacterial reduction. Therefore, temperature and silver release

percentage are key parameters in the antimicrobial activity of the dressing.

Analysis of Results for Low Concentration and 24-Hour Duration

The Pearson correlation coefficient between temperature and bacterial reduction in this condition was r = 0.206, indicating a weak positive correlation. Higher temperatures tended to slightly improve bacterial reduction, but the relationship was not strong. The Pearson correlation coefficient between silver release percentage and bacterial reduction was r = 0.397, indicating a moderate positive correlation. Higher silver release percentages tended to improve bacterial reduction, although the relationship was not particularly strong.

The weak positive correlation between temperature and bacterial reduction suggests that temperature slightly influences the effectiveness of the silver nanocrystal dressing, although other factors may also significantly contribute. The moderate positive correlation between silver release percentage and bacterial reduction indicates that greater silver release tends to improve bacterial reduction, but the relationship is not very strong. Additionally, the irregular results at 26 °C and 29 °C may suggest the presence of an optimal temperature range for the dressing's efficacy.

Analysis of Results for High Concentration and 24-Hour Duration

The Pearson correlation coefficient between temperature and bacterial reduction in this condition was r = 1.088, indicating a strong positive correlation. Higher temperatures significantly improved bacterial reduction. The Pearson correlation coefficient between silver release percentage and bacterial reduction was r = 0.370, indicating a moderate positive correlation. Higher silver release percentages tended to enhance bacterial reduction, though not very strongly.

The strong positive correlation between temperature and bacterial reduction confirms that elevated temperatures boost the antimicrobial efficacy of the silver nanocrystal dressing. Meanwhile, the moderate positive correlation between silver release percentage and bacterial reduction indicates that higher silver release improves bacterial reduction, albeit to a limited extent. Additionally, the irregular pattern observed at 32 °C suggests that there may be an optimal temperature range for maximizing dressing efficacy.

4. Discussion and Conclusion

The present study investigated the impact of environmental temperature on the antibacterial efficacy and silver ion release of Agicoat© silver nanocrystal dressings in response to *Staphylococcus aureus* under varying thermal and microbial load conditions. The findings offer novel insights into the interplay between temperature and nanoparticle-based wound dressings, revealing that both the bacterial reduction rate and silver ion release are influenced by thermal shifts. Specifically, elevated temperatures (above 30 °C) enhanced the antibacterial performance of Agicoat©, particularly in high bacterial load settings and over extended durations (24 hours), whereas in low bacterial load and shorter exposure scenarios, temperature had a more limited or variable influence.

In conditions of low bacterial concentration and 8-hour incubation, a weak positive correlation (r = 0.173) was found between temperature and bacterial reduction, while a moderate negative correlation (r = -0.381) was observed between silver release and bacterial reduction. These results suggest that while higher temperatures may marginally enhance bacterial killing, silver ion release at lower microbial densities does not necessarily result in proportionate bacterial reduction. This aligns with prior findings indicating that nanoparticle action is not solely dependent on dosage but also on dynamic microbial interactions and environmental factors [8]. In fact, Qu et al. emphasized that excessive silver ion presence under certain metabolic conditions may disrupt microbial ATPase activity in a nonlinear fashion, leading to metabolic adaptation rather than eradication [13].

Contrastingly, in the case of high bacterial load and 8-hour incubation, strong positive correlations were identified between both temperature and bacterial reduction (r = 1.436), and silver release and bacterial reduction (r = 0.898). These findings indicate that both thermal increase and enhanced silver ion availability synergistically contribute to microbial suppression when the bacterial challenge is intense. This supports studies highlighting the dose-dependent efficacy of silver nanoparticles in biologically challenging environments [1, 3]. Moreover, the antimicrobial synergy between silver ion concentration and temperature has been previously proposed in biosynthesis-based formulations, where thermal modulation improved surface reactivity and oxidative stress at the nanoparticle interface [14].

In low concentration and 24-hour conditions, the relationship between temperature and bacterial reduction remained weakly positive (r = 0.206), while the correlation

between silver release and bacterial reduction was moderately positive (r = 0.397). This indicates that extended exposure time may not significantly enhance bacterial killing in low-density infections unless combined with sufficient silver ion concentration. These observations are consistent with early experimental data showing that the mere prolongation of exposure, without optimal environmental stimuli, does not yield substantial microbial suppression [9]. Furthermore, the non-linear or irregular outcomes observed at mid-range temperatures (26–29 °C) suggest that there might exist a threshold or optimal thermal zone for maximizing Agicoat©'s effectiveness, echoing findings by Cuthbertson and Tilstone who identified specific temperature ranges conducive to accelerated epithelial regeneration in rodent models [10].

In high concentration and 24-hour exposure, the correlation between temperature and bacterial reduction was again strong (r = 1.088), while the relationship between silver release and bacterial reduction was moderate (r = 0.370). These findings validate the notion that thermal elevation remains a critical enhancer of Agicoat© performance even under high microbial stress. Notably, the slight decline in correlation strength for silver release compared to the 8-hour test may reflect a saturation effect or adaptive microbial mechanisms mitigating the extended silver exposure. This resonates with the work of Noga et al., who argued that the cytotoxic and antimicrobial activities of AgNPs plateau over time and may trigger bacterial resistance pathways if not paired with environmental modulation [15].

Collectively, these findings highlight the dual dependency of Agicoat© performance on both environmental temperature and microbial load intensity. They reinforce the central claim that the thermal conditions under which silver nanocrystal dressings operate are not mere background variables but active determinants of antimicrobial efficacy. This echoes research by McGuiness and colleagues, who demonstrated that dressing-related fluctuations in wound temperature can significantly affect local healing kinetics [6]. More recently, Zhu et al. underscored the role of localized thermal regulation in wound treatment, arguing that optimal healing occurs within a narrow thermal envelope, deviations from which may compromise the host's immune responses and nanoparticle functionality [8].

The release of silver ions, while generally associated with increased antibacterial activity, showed context-dependent behavior in this study. In high bacterial load environments, silver ion release correlated strongly with bacterial reduction, whereas in low bacterial load conditions, the relationship was weak or even negative. This suggests a complex interplay between microbial density, silver ion bioavailability, and environmental temperature. According to Harun-Ur-Rashid et al., silver-based polymer nanocomposites should be designed with responsiveness to such variables in mind, allowing for adaptive release mechanisms based on local environmental stimuli [2]. Agicoat©, as a nanocrystal dressing, could benefit from such modular design strategies that tune ion release based on bacterial load and ambient temperature.

It is also important to situate these findings within the broader technological and clinical context. The green synthesis of silver nanoparticles, as advocated by Aldakheel et al., emphasizes not only antimicrobial effectiveness but also environmental safety and biocompatibility, especially when embedded in hydrogel platforms [16]. The therapeutic potential of AgNPs in wound care continues to be validated across dermatological, orthopedic, and dental applications [4, 17, 21]. However, as studies such as those by Federica Paladini have pointed out, the translation of laboratory efficacy into clinical outcomes remains contingent on optimizing delivery conditions—including temperature, pH, and mechanical stress—within the wound bed [22].

Ultimately, this study contributes to a growing body of evidence calling for the reconsideration of ambient temperature in wound care protocols, especially when using advanced nanomaterial-based dressings. The current paradigm, which often treats temperature as a static or secondary variable, may be insufficient when dealing with dynamic systems like AgNPs that respond actively to their surroundings. The integration of thermally responsive feedback systems, whether in the form of smart dressings or externally regulated incubators, could mark a new era in precision wound care—one that not only kills pathogens effectively but also accelerates healing while minimizing systemic toxicity [12].

Limitations

Despite its valuable contributions, this study has several limitations. First, it was conducted under controlled laboratory conditions, which may not fully reflect the complex physiological dynamics of actual human wounds. Second, only one bacterial species (*Staphylococcus aureus*) was examined, limiting the generalizability of the findings to polymicrobial infections or fungal biofilms. Third, while silver ion release was measured as a proxy for dressing

efficacy, other influential factors such as oxidative stress generation, local tissue response, or nanoparticle degradation were not assessed. Additionally, temperature control was conducted externally through incubators, which does not fully replicate the variable and sometimes unstable thermal environment of a real wound site.

Suggestions for Future Research

Future studies should explore the effectiveness of silver nanocrystal dressings across a broader spectrum of microbial agents, including drug-resistant bacteria and fungal pathogens. Investigations should also incorporate in vivo models to simulate real wound healing conditions, allowing assessment of immune response, tissue regeneration, and systemic effects. Moreover, integrating sensor-based technologies to monitor real-time temperature and ion release at the wound site could offer valuable insights into dynamic therapeutic feedback. Further, comparative studies with other nanomaterials or green-synthesized composites may reveal superior formulations or synergistic combinations. Finally, long-term studies assessing resistance development, especially under prolonged exposure to silver nanoparticles, would provide a more holistic understanding of safety and efficacy.

Suggestions for Practice

Clinicians and healthcare practitioners should consider ambient temperature as a modifiable variable that can influence the success of nanomaterial-based wound treatments. Incorporating adjustable temperature control in wound care settings, especially for chronic or infected wounds, may improve treatment outcomes. Hospitals could explore protocols that align room or localized wound temperatures with the thermal activation range of advanced dressings like Agicoat©. Moreover, manufacturers of wound care products should invest in the development of responsive materials that can adapt to environmental changes, particularly temperature. This approach has the potential to reduce healing times, minimize complications, and optimize the use of healthcare resources.

Authors' Contributions

Authors equally contributed to this article.

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Declaration of Interest

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Ethical Considerations

All procedures performed in this study were under the ethical standards.

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