ASEAN Journal for Science and Engineering in Materials

Journal homepage: https://ejournal.bumipublikasinusantara.id/index.php/ajsem

Film-Forming Chitosan/Poly (vinyl alcohol)–Poly (hexamethylene biguanide) Antiseptic Spray for Wound Cleansing: A Sustainable Healthcare Approach Aligned with Sustainable Development Goals (SDGs) Completed with Bibliometric Analysis

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ABSTRACT

Proper wound cleansing is a fundamental step in preventing infection and supporting effective wound management. This study aimed to develop and evaluate a film-forming antiseptic spray composed of chitosan, polyvinyl alcohol, and polyhexamethylene biguanide for wound cleansing applications. The formulation was prepared using a solution-based approach and characterized in terms of morphology, chemical interactions, sprayability, film formation stability, antibacterial, and antibiofilm performance. The developed spray demonstrated uniform application, stable film formation, and effective incorporation of the antimicrobial agent. Biological evaluations confirmed enhanced antibacterial and antibiofilm activity because of synergistic interactions between the biopolymer matrix and the cationic antiseptic component. This study was further supported by bibliometric evidence indicating a growing global research focus on wound care materials and film-forming antiseptic sprays, emphasizing their relevance to sustainable healthcare innovation. The findings contribute to the advancement of sustainable wound care technologies and align with the Sustainable Development Goals, particularly the goal of promoting good health and well-being.

ARTICLE INFO

Article History:
Submitted/Received 02 Aug 2025
First Revised 29 Oct 2025
Accepted 21 Jan 2026
First Available online 22 Jan 2026
Publication Date 01 Dec 2026

Keyword:
Antibacterial,
Anti-biofilm,
Antiseptic spray,
Chitosan,
PHMB,
Wound cleansing.

1. INTRODUCTION

Microbial invasion, colonization, and subsequent proliferation at the wound site lead to wound infections (Malone & Schultz, 2022). A moist environment can increase microbial infiltration at wound sites and can upregulate various pathological infections. Some of the most commonly found microorganisms at the wound site include *E. coli*, *P. aeruginosa*, *S. aureus*, MRSA, *Klebsiella spp*, *Bacteroides spp.*, and *Peptostreptococcus spp* (Negut *et al.*, 2018; Jiang *et al.*, 2023). These microbes actively proliferate and cause polymicrobial infections, resulting in delayed wound healing (Antony *et al.*, 2023).

Wound cleansing with an appropriate antiseptic is the first step involved in wound management and is focused on reducing bacterial load at the wound site (Wilkins & Unverdorben, 2013). This step is essential because reducing the bacterial load is pivotal in speeding up the healing process. Out of many topical dosages and formulations, sprays have added advantages such as uniform distribution of the bioactives, ease of use, maintaining sterility, low irritability, and even coverage of the wound site (Umar *et al.*, 2020). There are different categories of topical antiseptics that effectively help in the inhibition of microbes and control infection progression at the wound site. Povidone iodine, chlorhexidine (biguanide), hydrogen peroxide, acetic acid, triclosan (bisphenol), alcohols, and Dakin's solution are some of the currently used topical solutions (Atiyeh *et al.*, 2009). The major disadvantages of these conventional topical solutions are poor adherence, lack of patient compliance, and the lack of longer contact of the bioactives at the infected area (Kathe & Kathpalia, 2017). Thus, film-forming topical sprays act as an alternative for conventional sprays, where they form thin films upon spraying, which can increase the permeability and contact time of the bioactive at the wound site. This can effectively increase the efficiency of the spray formulation and can also be uniformly applied to wounds of any shape, depth, and size (Chamsai *et al.*, 2022).

Chitosan (CS) is a biopolymer with *D*-glucosamine and *N*-acetyl-*D*-glucosamine units, sourced from deacetylating chitin, which is obtained from fungal cell walls and exoskeletons of crustaceans (Patrulea *et al.*, 2015). This cationic polymer has a copious amount of applications in the field of biomedicine, substantially due to its biocompatibility, non-toxicity, and biodegradability (Ahmed & Ikram, 2016). Chitosan's ability to promote wound contraction, its mucoadhesiveness, antioxidant, antibacterial, anti-inflammatory, and hemostatic properties make chitosan a quintessential biopolymer (Aranaz *et al.*, 2021; Liu *et al.*, 2018). The electrostatic interaction between the chitosan and anionic bacterial cell wall leads to cell disruption and death (Dai *et al.*, 2011; Matica *et al.*, 2019). The synthetic hydrophilic polymer Poly (vinyl alcohol) (PVA) is utilized widely in the biomedical field, owing to its large number of properties like biodegradability, great biocompatibility when mixed with other biopolymers, chemical stability, transparency, mechanical properties, non-toxicity, and non-carcinogenicity (Ma *et al.*, 2017; Oun *et al.*, 2022). To go with, this polymer has gained much interest due to its film-forming and adhesive properties (Rahman Khan *et al.*, 2024; Liu *et al.*, 2022; Wang *et al.*, 2014; Yang *et al.*, 2008). In the development of wound dressings, drug release systems, tissue scaffolding, adhesion industry, food packaging, and filtration membranes, where PVA is greatly used (Guzman-Puyol *et al.*, 2015). PHMB (Polyhexanide) is a broad-spectrum antiseptic agent used in topical formulations, which is composed of hexamethylene connected biguanide units (Mulder *et al.*, 2007; Wang *et al.*, 2023; Chindera *et al.*, 2016). The positive charge of PHMB enables its binding to bacterial cell walls. The mechanism of action of PHMB is such that it interacts with the cytoplasmic membrane of the bacteria, which incites the complexation with the phospholipids. This brings

about the outflow of cytoplasmic components, disruption of osmotic equilibrium, and bacterial death (Dias *et al.*, 2021; Sowlati-Hashjin *et al.*, 2020).

In this study, we have developed an antiseptic CS/PVA-PHMB spray with film-forming ability, stability, good spray angle, antibacterial, and anti-biofilm properties that can be used for wound cleansing, as shown in **Figure 1**. In addition, this study was completed with a bibliometric analysis to show the reason why this study is important. In this regard, the present study aligns with the Sustainable Development Goals (SDGs), especially the goal of promoting good health and well-being through accessible and innovative healthcare technologies. Therefore, this study aims to develop and evaluate a film-forming chitosan/poly(vinyl alcohol)–poly(hexamethylene biguanide) antiseptic spray with antibacterial and anti-biofilm properties for wound cleansing applications.

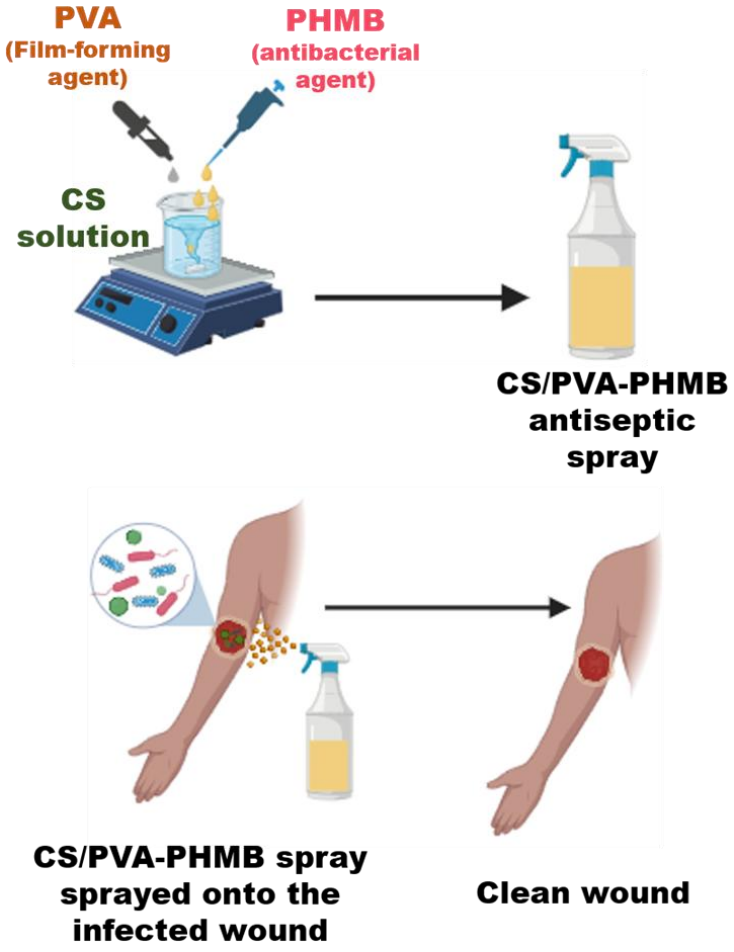

Figure 1. Schematic representation of the antiseptic action of the CS/PVA-PHMB spray.

2. METHODS

2.1. Materials

Chitosan (Avg MW: 100-150kDa, DD: 85%, Japan, Koyo Chemicals Ltd), Poly (vinyl alcohol) (98%, typical MW: 13,000-23,000, Sigma, USA), Glacial acetic acid (MW: 60.05, Spectrochem, India), 20% Poly (hexamethylene)biguanide solution (Simson Pharma Ltd, India), Sodium bicarbonate (HiMedia, India), Luria Bertani broth, and Agar (HiMedia, India) were purchased. Bacterial strains of *E. coli* (ATCC 25922), *S. aureus* (ATCC 25923), and their clinical isolates were obtained from the Microbiology Laboratory of Amrita Institute of Medical Sciences, Cochin, India.

2.2. Methods

2.2.1. Preparation of chitosan spray (CS/PVA spray)

CS/PVA spray solution was prepared following the procedure below. 1.5% Chitosan solution in 100 mL was prepared by stirring chitosan powder in 1% acetic acid solution. The pH of the solution was adjusted to 6.5 by adding sodium bicarbonate. 5% poly(vinyl alcohol) (PVA) was added in the ratio 5:1 (CS:PVA) to this and kept stirring. The obtained solution is the CS/PVA spray solution (**Figure 2**).

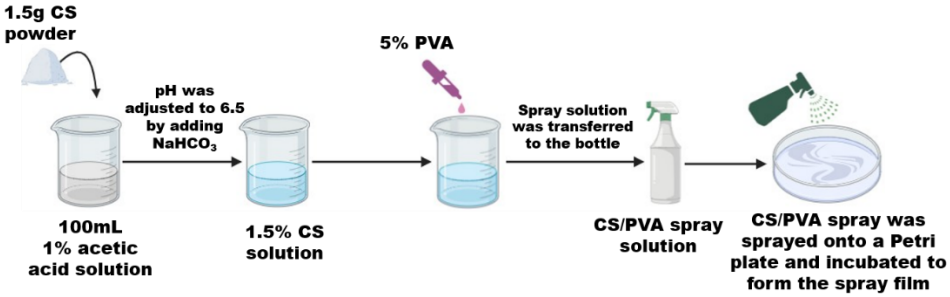

Figure 2. Preparation of CS/PVA spray solution.

2.2.2. Preparation of chitosan/PVA-PHMB spray (CS/PVA-PHMB spray)

Initially, the CS/PVA spray solution was prepared as explained. To 98 mL of this solution, 2 mL of PHMB was added and thoroughly stirred to obtain the chitosan/PVA -PHMB spray (CS/PVA -PHMB) as shown in **Figure 3**.

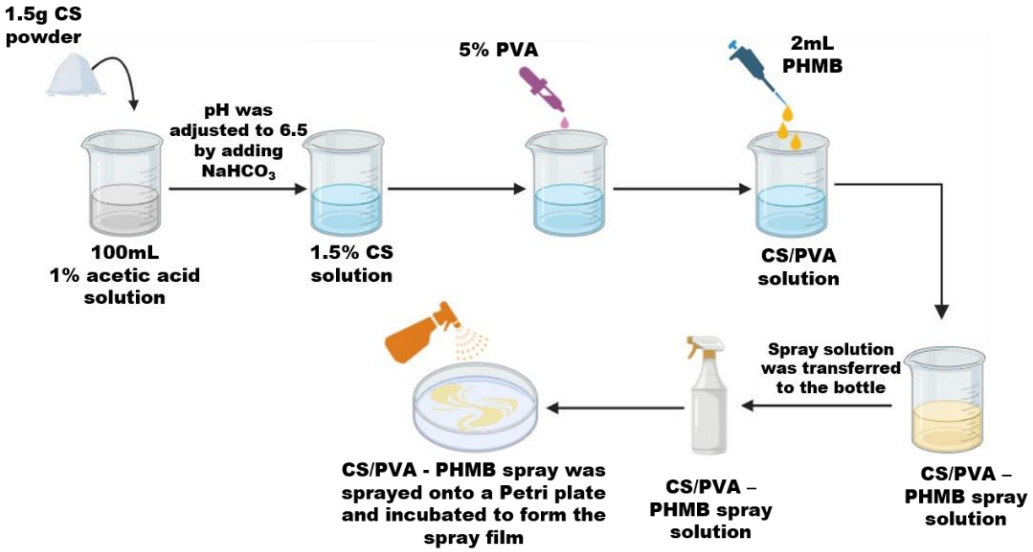

Figure 3. Preparation of CS/PVA -PHMB spray.

2.2.3. Characterization of prepared CS/PVA and CS/PVA-PHMB spray

The morphology of the prepared CS/PVA and CS/PVA-PHMB spray was characterized using Scanning Electron Microscopy (SEM). The gold-sputtered samples (JEOL, JFC-1600) were scanned at an acceleration voltage of 15 kV using JEOL JSM-6490LA. Further characterization of the spray samples was carried out by analysing the Fourier Transform Infrared spectroscopy (Shimadzu, Japan). The spray samples were lyophilized and powdered to get the samples for FTIR analysis.

2.2.4. Spray Angle of the prepared spray

The spray solutions were further characterized by determining the spray angles. By following the previously reported protocols with slight modification, we assessed the spray angle of the solutions (Shetty *et al.*, 2024; Soujith & Jawahar, 2023). The CS/PVA and the CS/PVA-PHMB spray bottles were placed 15 cm (*d*) away from a filter paper, and the solutions were sprayed to observe the patterns. Post-spraying, the diameter of the patterns that had formed on the filter paper was measured. The spray angle was calculated using the formula: Spray angle (ϑ) = $\tan^{-1}(d/r)$, where *r* is the radius of the pattern formed, and *d* is the distance between the nozzle of the spray bottle and the filter paper.

2.2.5. Thickness of the spray films

The film thickness of the prepared CS/PVA and CS/PVA-PHMB spray solutions was assessed by spraying them onto a Petri plate, followed by drying at 37°C. The thickness of the obtained films was measured using a Mitutoyo screw gauge (resolution = 0.001 mm). Multiple points on the same film were evaluated, and the average value was calculated to determine the final film thickness.

2.2.6. Stability of the prepared spray

The stability of the prepared spray solutions upon storage was evaluated to determine their efficacy and stability in different temperature settings (Mori *et al*., 2017). The CS/PVA and CS/PVA-PHMB spray solutions were stored at three different temperature conditions: refrigerator (at 4ºC), room temperature (at 25°C), and incubator (at 37ºC) for 14 days. The samples were subsequently analysed for any physical changes.

2.2.7. Antibacterial activity of the CS/PVA-PHMB spray

The antibacterial activity of the prepared CS/PVA-PHMB spray was evaluated against ATCC strains and clinical isolates of *E. coli and S. aureus* using the agar well diffusion method (Sritharadol *et al*., 2017). CS/PVA spray was kept as the control. Inoculation of the bacterial cultures was prepared by adding 50 µL of the respective bacteria to 5 mL of Luria Bertani (LB) broth on the previous day of the experiment. On the experiment day, LB plates were uniformly spread with 50 µL of the respective bacteria using a cotton swab. Two wells were created in each plate, and 70 µL of CS/PVA and CS/PVA-PHMB spray were added to these wells. The plates were incubated for 24 hours at 37ºC, and the ZOI was measured. Similarly, activity against clinical isolates was evaluated using five different clinical isolates of *E. coli* and *S. aureus*. The plates were streaked with the respective isolates, and wells were created in the centre of each streak. A volume of 70 µL of the CS/PVA-PHMB spray was added to the wells and incubated for 24 hours. Zones of inhibition were observed after 24 hours of incubation.

2.2.8. Anti-biofilm activity of the CS/PVA-PHMB spray

The *in vitro* anti-biofilm activity of the prepared sprays against *E. coli* and *S. aureus* was determined to analyze the ability of the spray solutions in eradicating the respective bacterial biofilms. The protocol was adapted from literature (Mothilal *et al*., 2024) with modifications. Onto the sterile coverslips placed inside the wells of a 12-well plate, *E. coli* and *S. aureus* were cultured in 1 mL LB broth and 80 µL of 2% glucose. After 48 hours of incubation at 37ºC, the previous cultures were discarded, and a fresh batch of LB broth and glucose solutions was

added to these wells. Among the wells, one set of wells was kept as bacterial control, one set was provided with 100 mg of 2% CS gel mixed with 70 µL of CS/PVA-PHMB spray, one set with 100 mg of 2% CS gel mixed with 140 µL of CS/PVA-PHMB spray, and one set with 100 mg of 2% CS gel mixed with 70 µL of CS/PVA spray solution. This again was incubated for 24 hours at 37ºC. The coverslips were washed with phosphate buffer solutions (PBS) and dried overnight. The next day, the coverslips were stained with 0.1% acrydine orange for 5 minutes, washed with PBS, and again dried. The coverslips were imaged using a fluorescence microscope, and ImageJ software was used to examine the qualitative inhibition of the biofilms. For quantitative analysis of biofilm inhibition, the coverslips were immersed in 70% acetone overnight, and the fluorescence intensity was measured at 525 nm for emission and 400 nm for excitation using a microplate reader.

2.2.9. In vivo antibacterial study of CS/PVA-PHMB spray on Drosophila melanogaster model

Banana fly culture medium maintained at 25ºC (room temperature) was used to raise and maintain *Drosophila melanogaster* stocks. The culture was prepared by mixing 3 g of agar, 40 g of banana paste (prepared by blending ripe banana), 25 mL of water, and boiling them together for 1 minute. Upon requirement, water was further added and boiled for another 1 min to get a runny consistency. This was then poured into glass vials secured with cotton plugs. The *in vivo* antibacterial study was then performed by anesthetizing the flies (2-5 days old) by keeping them on ice for 1-2 minutes. A 25-gauge needle dipped in *E. coli* and *S. aureus* cultures was used to infect the flies by pricking them in the dorsal thorax region. The flies were then returned to the glass vials (10 flies per glass vial) and fed with CS/PVA and CS/PVA-PHMB spray solution. The fly survivability was observed for the next 12 hours at 0, 3, 6, and 12-hour intervals. The percentage of flies alive at each time point after infection was plotted to analyse the result (Nimal *et al.*, 2016).

3. RESULTS AND DISCUSSION

3.1. Preparation and characterization of film-forming CS/PVA-PHMB spray

A 1.5% CS solution was prepared by dissolving CS in 1% acetic acid solution. $NaHCO_3$ was added to the above solution to adjust the pH of the solution to 6.5. The 5% PVA in the formulation, which was added to the CS solution in the ratio 5:1, imparts the film-forming ability to the resultant CS/PVA spray solution (**Figure 4A**). This prepared CS/PVA spray solution was used as the control throughout the study. The CS/PVA-PHMB spray solution was prepared by adding 2 mL of the PHMB solution to the CS/PVA spray solution (**Figure 4A**). These spray solutions exhibited excellent spraying ability (**Figure 4B**) and formed thin films upon spraying and drying (**Figure 4C).**

Further characterization of the CS/PVA-PHMB spray solution using SEM revealed the droplet nature of the CS/PVA-PHMB spray solution. The droplet size ranged from 500 to 1000 µm and showed smooth morphology as shown in **Figure 4D**. Additionally, the CS/PVA and CS/PVA-PHMB spray solutions were characterized by the FTIR technique (**Figure 4E**). The spray samples were lyophilized and powdered to obtain the samples for FTIR analysis. Characteristic peaks of CS at 1662 and 1329 cm^{-1} (corresponding to amide group) (Mothilal *et al.,* 2024), and 1167 cm^{-1} (corresponding to glycosidic linkage) (Pandian *et al.*, 2021) were obtained for both CS/PVA and CS/PVA-PHMB spray samples. Characteristic peaks of PHMB corresponding to nitrogen-related vibrations at 2363, 1630, and 1548 cm^{-1} (corresponding to –NH bending of imine groups of PHMB) (Pandian *et al.*, 2021) were observed for the CS/PVA-PHMB. This implied the successful incorporation of PHMB in the CS/PVA-PHMB formulation. Detailed information regarding the use of FTIR is explained elsewhere (Nandiyanto, 2026).

3.2. Spray angle of the spray

Spray angle of a solution is an important parameter that helps us understand the spray behaviour, properties, and their ability to be used in various topical applications. Spray angles of the CS/PVA and CS/PVA-PHMB spray solutions were calculated by measuring the diameter of the spray pattern formed by these solutions. Using the measurement of the radii, the average spray angles obtained for CS/PVA and CS/PVA-PHMB spray were 72.12 ± 1.806 and 82.357 ± 0.357º, respectively (**Table 1**). The values suggest that the spray angle of the CS/PVA-PHMB spray is higher than that of the control spray. It is reported in literature that a spray angle below 85º is considered a good spray angle (Umar *et al.*, 2021), and hence, according to this, both CS/PVA and CS/PVA-PHMB spray solutions showed good spray angles.

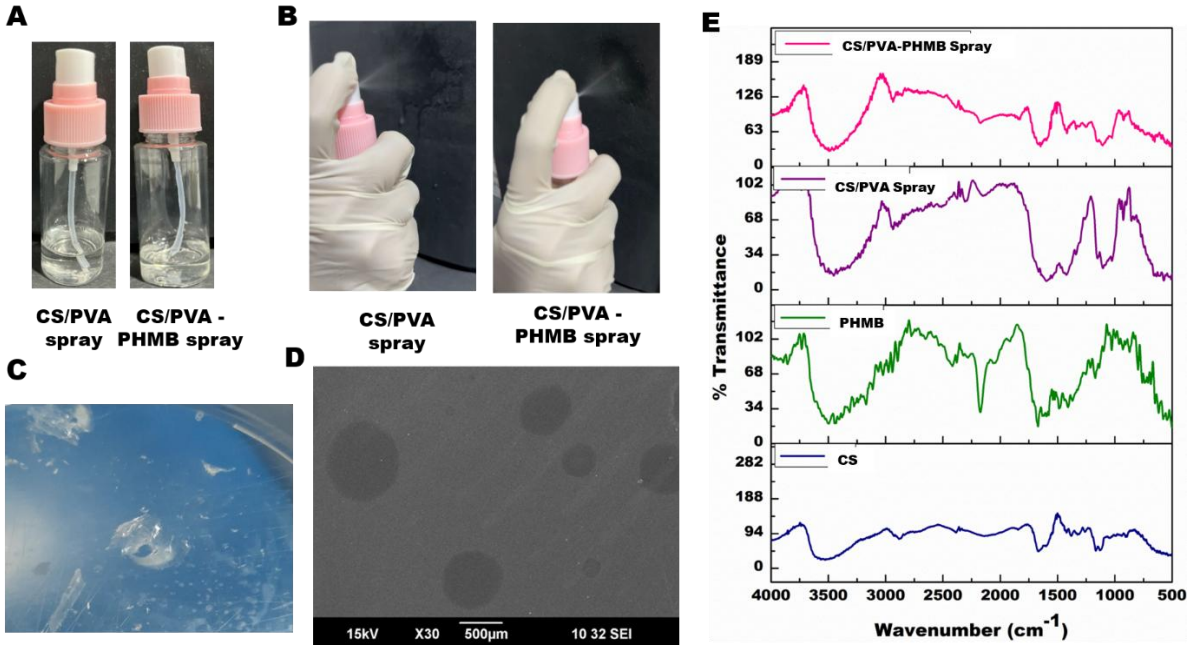

Figure 4. (A) Prepared CS/PVA and CS/PVA-PHMB spray, (B) Sprayability of CS/PVA and CS/PVA-PHMB spray, (C) Photographic image of the CS/PVA-PHMB spray film, (D) SEM image of the CS/PVA-PHMB spray solution, and (E) FTIR analysis of CS/PVA and CS/PVA-PHMB spray.

Table 1. Spray angles of CS/PVA and CS/PVA-PHMB spray.

Spray	Angle in degrees (º)	Angle in radians (rad)
CS/PVA spray	72.12 ± 1.806	1.25 ± 0.032
CS/PVA-PHMB spray	82.357 0.357	1.43 ± 0.006

3.3. Evaluation of the film-forming ability of CS/PVA and CS/PVA-PHMB spray

The ability to form films upon spraying is a desired property in antiseptic sprays, because this will enhance the contact time of the bioactive with the infected wound site. When such sprays are used, the films can form around any wounds of irregular size, shape, and depth, and thereby increase the efficiency of the spray. In this study, PVA was included in the formulation to impart film-forming ability to the spray solutions. The control CS/PVA as well as the study group CS/PVA-PHMB solution exhibited good film-forming ability when the sprayed solutions were allowed to dry by incubating them at 37ºC. The films thus formed

were peeled off from the Petri plates, and the thickness of the film at different points of the film was measured using a screw gauge. The film thickness of the CS/PVA and CS/PVA-PHMB spray solutions was found to be 0.35 ± 0.009 and 0.076 ± 0.027 mm, respectively (**Table 2**). This indicates that the CS/PVA-PHMB spray produced a slightly thicker film when compared to the CS/PVA spray. The film-forming ability of the prepared spray solution can aid in enhanced contact time of the antibacterial agent in the formulation at the wound site and thereby increase the efficiency of microbial elimination and wound cleansing.

Table 2. Film thickness of CS/PVA and CS/PVA-PHMB spray.

Spray	Thickness of the film(mm)			Average (mm)
CS/PVA spray	0.046	0.032	0.028	0.35 ± 0.009
CS/PVA-PHMB spray	0.086	0.097	0.045	0.76 ± 0.027

3.4. Stability of the prepared sprays

The ability to be stored without precipitating out the components and/or contamination is an important parameter while formulating a spray solution. The stability of the prepared sprays was evaluated by storing them at three different temperatures (4ºC - refrigerator, 25ºC - room temperature, and 37ºC - incubator) for 14 days. The spray solutions remained stable, with no signs of contamination or precipitation throughout the study period (**Figure 5**). This implies that the spray formulation is stable at these temperatures and can be stored for at least 14 days without forming any precipitates.

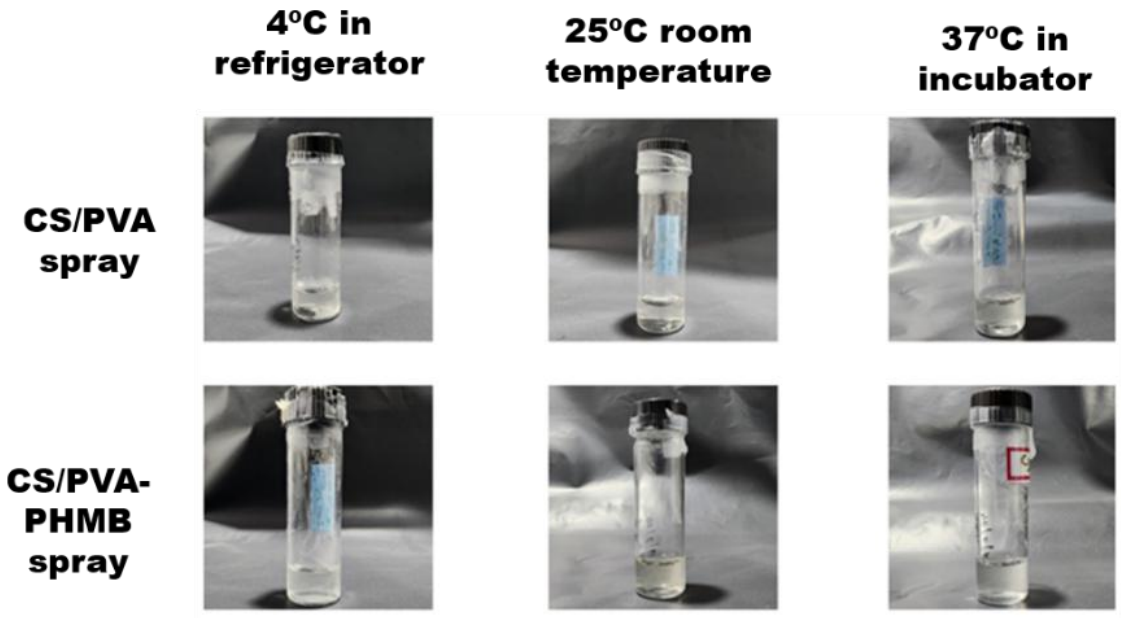

Figure 5. Stability test of CS/PVA and CS/PVA-PHMB spray.

3.5. Antibacterial activity of the CS/PVA-PHMB spray

The antibacterial activity of the CS/PVA-PHMB spray against gram-negative *E. coli* and gram-positive *S. aureus* was investigated by conducting the agar well diffusion method. The CS/PVA spray served as the control. The resulting zones of inhibition (ZOI) indicated the ability of the spray solutions to inhibit bacterial growth. A larger ZOI was observed around the CS/PVA-PHMB spray when compared to the control CS/PVA spray (Figure 6A). The measured zones for CS/PVA-PHMB spray against *E. coli* and *S. aureus* were 18 and 24 mm, respectively.

This clearly indicates that the incorporation of PHMB into the spray formulation improved its bacterial growth inhibition efficiency. This improved and enhanced antibacterial activity could be possibly due to the binding of the PHMB to the bacterial phospholipid layers, which increases the membrane permeability, leading to leakage of cytoplasmic constituents and eventually resulting in bacterial death (Antony *et al.*, 2023). To further confirm the results, the prepared spray solution was tested against five different strains of clinical isolates of *E. coli* and *S. aureus*, and the results supported the previous observation, which points towards the improved effectiveness and antibacterial efficiency of the spray, as shown in **Figure 6B**. In short, the CS/PVA-PHMB spray showed enhanced antibacterial activity compared to the CS/PVA spray by inhibiting the growth of both ATCC strains and clinical isolates of the tested bacteria. This indicates the potential of the CS/PVA-PHMB spray in effectively cleansing the microbial-contaminated wound sites.

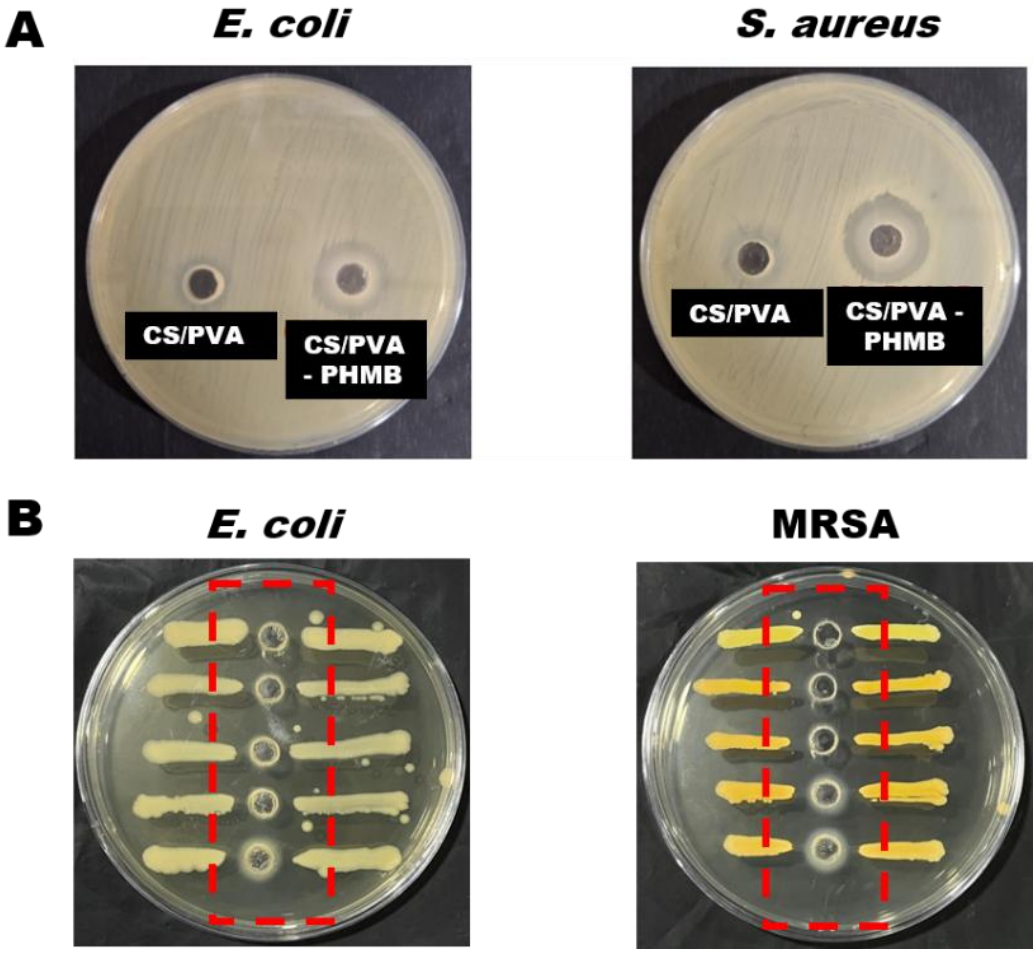

Figure 6. (A) *In vitro* antibacterial study of CS/PVA-PHMB spray against the ATCC strains of *E. coli*, *S. aureus,* and (B) antibacterial study of CS/PVA-PHMB spray against the clinical isolates of *E. coli*, *S. aureus*.

3.6. Anti-biofilm activity of the CS/PVA-PHMB spray

At the wound/infection site, microorganisms can exist in two phenotypic states: as free-living organisms (planktonic) or as biofilms. The free-living microorganisms can adhere to favourable surfaces and grow into biofilms, where they are protected by polymeric matrices and thereby adapt to survive (Sen *et al.*, 2021; Anju *et al.*, 2022). Thus formed biofilms have a significant influence on the host inflammation in the cases of chronic infections (Hurlow *et*

al., 2025). Therefore, targeted treatment strategies to remove these biofilms are very important in wound treatment and management. For the qualitative analysis of the biofilm study, the coverslips stained using acridine orange were observed through a fluorescence microscope, and the images obtained were converted using ImageJ software. High fluorescence intensity indicates low biofilm inhibition, whereas low fluorescence intensity indicates high biofilm inhibition. The coverslips treated with CS/PVA-PHMB spray showed low fluorescence, which indicates that the spray solutions were able to disrupt the biofilm (**Figure 7A**). The biofilm reduction was further quantified using the readings from the microplate reader. For *E. coli*, the fluorescence values for bacterial control, CS/PVA spray, CS/PVA-PHMB spray (70µL), and CS/PVA-PHMB spray (140µL) were 30696 ± 4671, 24837 ± 5173, 16965 ± 2242, and 8386 ± 635 RFUs, respectively, whereas for *S. aureus*, the values were 32049 ± 217, 26223 ± 5071, 21598 ± 3191, and 13001 ± 1008 RFUs, respectively (**Figure 7B**). The reduction in fluorescence values indicates that the spray solution was effective in disrupting the biofilms of *E. coli* and *S. aureus.* These results suggest that no considerable biofilm inhibition was observed for the CS/PVA spray, whereas the CS/PVA-PHMB spray showed significant biofilm reduction. Also, when the volume of the CS/PVA-PHMB spray increased from 70 to 140 µL, an increase in biofilm reduction was observed. This anti-biofilm activity of the CS/PVA-PHMB spray can be attributed to the ability of PHMB to disrupt bacterial membranes by binding to them, resulting in cell death. Therefore, the CS/PVA-PHMB spray is a potential candidate as an antiseptic solution.

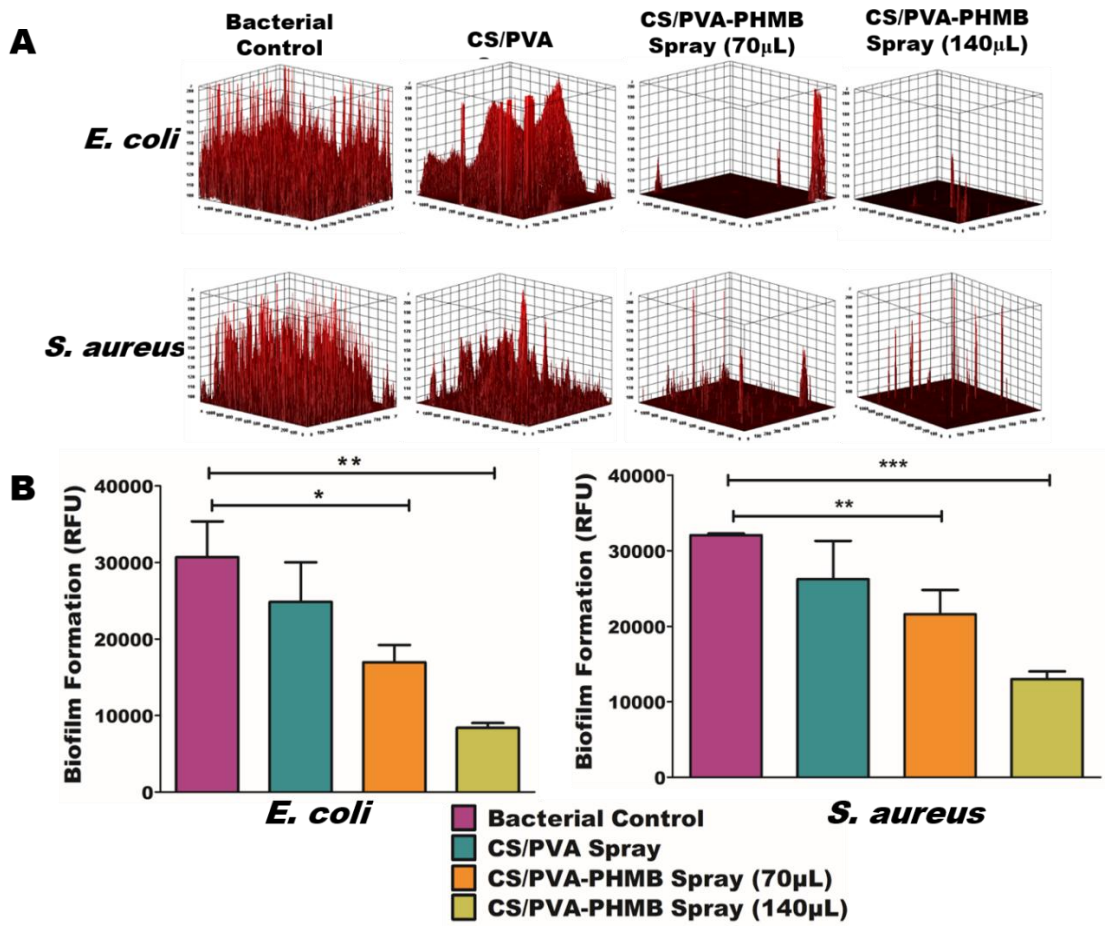

Figure 7. (A) 3D surface plots depicting biofilm inhibition in *E. coli* and *S. aureus*, and (B) quantification of biofilm reduction.

3.8. In vivo antibacterial study of CS/PVA-PHMB spray in Drosophila melanogaster fly model

The Drosophila fly model assists in evaluating the efficacy and effectiveness of PHMB in clearing out bacterial infection, based on the fly survival (Lee *et al.*, 2018; Cao *et al.*, 2023). In this study, the method of pricking (nicking) with a needle dipped in bacterial cultures was used to create the fly models (Mulcahy *et al.*, 2011). **Figure 8** shows the survival graph of *Drosophila melanogaster* when treated with the CS/PVA-PHMB spray solution. In the case of CS/PVA-PHMB spray, the *E. coli-infected* flies showed ≈ 60% fly survival after 12 hours, whereas for *S. aureus-infected* flies, it was ≈70%. More fly survival was observed for CS/PVA-PHMB spray when compared to CS/PVA spray and the infected group without treatment. The flies were able to survive the bacterial infection when food containing CS/PVA-PHMB spray solution was fed to them. This indicates the effectiveness of PHMB incorporation in the CS/PVA spray solution in surviving *E. coli* and *S. aureus* infections.

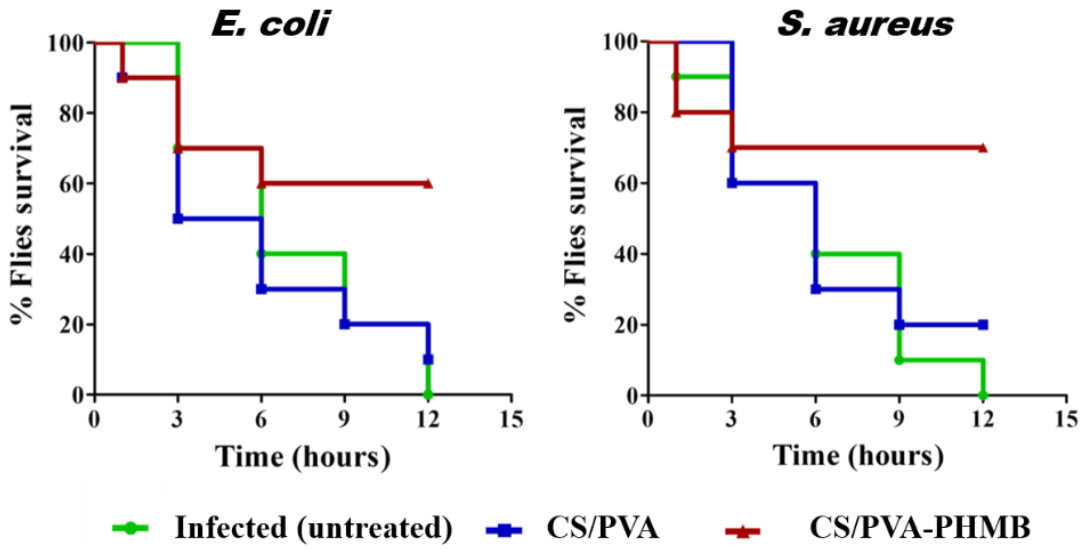

Figure 8. *In vivo* survivability graph of *Drosophila melanogaster* treated with CS/PVA and CS/PVA-PHMB spray.

3.8. Bibliometric Perspective on Wound Cleansing and Film-Forming Antiseptic Research

Figure 9 presents the bibliometric trend of Scopus-indexed publications retrieved using the TITLE-ABS-KEY query related to wound and cleansing. The temporal distribution indicates a clear and sustained increase in global research output over recent decades, with a pronounced acceleration in publications in the most recent years. This upward trend reflects the growing scientific and clinical attention toward wound management strategies, particularly those emphasizing infection prevention, cleansing technologies, and advanced topical formulations.

The increasing number of publications highlights a broad research interest in addressing wound-related challenges through innovative material-based and formulation-driven approaches. Despite this growth, a closer inspection of the literature suggests that many studies focus on conventional wound dressings, antiseptic solutions, or hydrogel systems, while comparatively fewer investigations explore film-forming antiseptic sprays designed specifically for wound cleansing applications. This observation underscores a research gap at the intersection of spray-based delivery systems, film-forming polymers, and broad-spectrum antiseptic agents.

Within this evolving research landscape, the present study contributes novel experimental evidence by integrating chitosan, poly(vinyl alcohol), and poly(hexamethylene biguanide) into a single film-forming antiseptic spray. The bibliometric trend, therefore, contextualizes the relevance of the developed formulation and supports its positioning within an emerging and rapidly expanding area of wound care research.

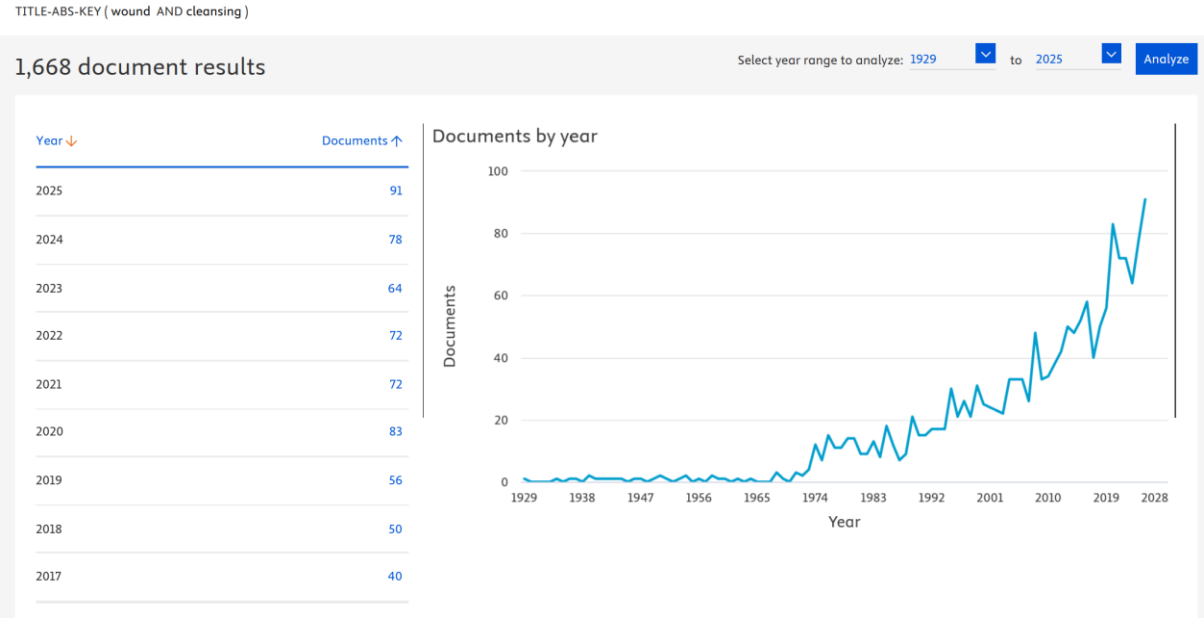

Figure 9. Bibliometric analysis using the keyword wound cleansing from scopus database taken in January 2026.

3.9. Implications of the Developed Antiseptic Spray for Sustainable Development Goals (SDGs)

Beyond its experimental performance, the developed film-forming chitosan/poly(vinyl alcohol)–poly(hexamethylene biguanide spray demonstrates relevance within the broader framework of sustainable healthcare. Wound infections remain a major burden on healthcare systems, particularly in resource-limited settings, where delayed healing and recurrent infections can significantly impact patient outcomes. In this context, effective wound cleansing technologies play a critical role in reducing infection risk and supporting recovery.

The antibacterial and antibiofilm performance observed in this study indicates that the proposed spray formulation has the potential to enhance infection control at the wound site through a simple and accessible application strategy. The film-forming nature of the spray further supports prolonged contact of the antiseptic agent with the wound surface, which may reduce the need for frequent reapplication and minimize treatment complexity. These characteristics are aligned with the objectives of the Sustainable Development Goals, particularly the goal of promoting good health and well-being by improving access to effective, safe, and affordable healthcare solutions. Moreover, the use of biopolymer-based components such as chitosan supports environmentally responsible material selection while maintaining functional performance. Collectively, the findings suggest that the developed antiseptic spray not only addresses immediate biomedical challenges but also contributes to sustainable and innovation-driven wound care strategies consistent with global health priorities.

4. CONCLUSION

This study successfully developed a film-forming chitosan/poly(vinyl alcohol)–poly(hexamethylene biguanide antiseptic spray with effective wound cleansing properties. The formulation demonstrated favorable sprayability, stable film formation, and enhanced antibacterial and antibiofilm activity, supported by both in vitro and in vivo evaluations. From a broader perspective, bibliometric trends indicate a growing global research focus on wound cleansing technologies and advanced antiseptic formulations, highlighting the relevance and timeliness of this work. Beyond its technical performance, the developed spray addresses critical needs in wound infection control through a simple and accessible application strategy. The use of biopolymer-based materials further supports sustainable and responsible healthcare innovation. Overall, the findings contribute to the advancement of effective wound care technologies and align with the SDGs, particularly the goal of promoting good health and well-being through improved infection prevention and management.

5. ACKNOWLEDGMENT

The authors are thankful to Amrita Vishwa Vidyapeetham, Kochi, India, for providing the infrastructure facility for this study.

6. AUTHORS' NOTE

The authors declare that there is no conflict of interest regarding the publication of this article. Authors confirmed that the paper was free of plagiarism.

7. REFERENCES

Ahmed, S., and Ikram, S. (2016). Chitosan based scaffolds and their applications in wound healing. *Achievements in the Life Sciences, 10*(1), 27-37.

Anju, V. T., Busi, S., Imchen, M., Kumavath, R., Mohan, M. S., Salim, S. A., Subhaswaraj, P., and Dyavaiah, M. (2022). Polymicrobial infections and biofilms: Clinical significance and eradication strategies. *Antibiotics, 11*(12), 1731.

Antony, I. R., Pradeep, A., Pillai, A. V., Menon, R. R., Kumar, V. A., and Jayakumar, R. (2023). Antiseptic chitosan-poly (hexamethylene) biguanide hydrogel for the treatment of infectious wounds. *Journal of Functional Biomaterials*, *14*(10), 528.

Aranaz, I., Alcántara, A. R., Civera, M. C., Arias, C., Elorza, B., Heras Caballero, A., Acosta, N. (2021). Chitosan: An overview of its properties and applications. *Polymers, 13*(19), 3256.

Atiyeh, B. S., Dibo, S. A., and Hayek, S. N. (2009). Wound cleansing, topical antiseptics and wound healing. *International Wound Journal, 6*(6), 420-430.

Cao, X., Scoffield, J., Xie, B., Morton, D. B., and Wu, H. (2023). Drosophila melanogaster as a model to study polymicrobial synergy and dysbiosis. *Frontiers in Cellular and Infection Microbiology, 13*, 1279380.

Chamsai, B., Soodvilai, S., Opanasopit, P., and Samprasit, W. (2022). Topical film-forming chlorhexidine gluconate sprays for antiseptic application. *Pharmaceutics, 14*(6), 1124.

Chindera, K., Mahato, M., Kumar Sharma, A., Horsley, H., Kloc-Muniak, K., Kamaruzzaman, N. F., Kumar, S., McFarlane, A., Stach, J., Bentin, T., and Good, L. (2016). The antimicrobial polymer PHMB enters cells and selectively condenses bacterial chromosomes. S*cientific Reports*, *6*(1), 23121.

Dai, T., Tanaka, M., Huang, Y. Y., and Hamblin, M. R. (2011). Chitosan preparations for wounds and burns: antimicrobial and wound-healing effects. *Expert Review of Anti-infective Therapy, 9*(7), 857-879.

Dias, F. G. G., de Freitas Pereira, L., Parreira, R. L. T., Veneziani, R. C. S., Bianchi, T. C., de Paula Fontes, V. F. N., de Carols Galvani, M., Cerce, D. D. P., Martins, C. H. G., Rinaldi-Neto, F., Ferreira, N. H., da Silva, L. H. D., de Oliveria, L. T. S., Esperandim, T. R., de Sousa, F. A., Ambrosio, S. R, and Tavares, D. C. (2021). Evaluation of the antiseptic and wound healing potential of polyhexamethylene guanidine hydrochloride, as well as its toxic effects. *European Journal of Pharmaceutical Sciences, 160*, 105739.

Guzman-Puyol, S., Ceseracciu, L., Heredia-Guerrero, J. A., Anyfantis, G. C., Cingolani, R., Athanassiou, A., and Bayer, I. S. (2015). Effect of trifluoroacetic acid on the properties of polyvinyl alcohol and polyvinyl alcohol–cellulose composites. *Chemical Engineering Journal*, 277, 242-251.

Hurlow, J., Wolcott, R. D., and Bowler, P. G. (2025). Clinical management of chronic wound infections: The battle against biofilm. *Wound Repair and Regeneration, 33*(1), 13241.

Jiang, P., Li, Q., Luo, Y., Luo, F., Che, Q., Lu, Z., Yang, S., Yang, Y., Chen, X., and Cai, Y. (2023). Current status and progress in research on dressing management for diabetic foot ulcer. *Frontiers in Endocrinology, 14*, 1221705.

Kathe, K., and Kathpalia, H. (2017). Film forming systems for topical and transdermal drug delivery. *Asian Journal of Pharmaceutical Sciences, 12*(6), 487-497.

Lee, Y. J., Jang, H. J., Chung, I. Y., and Cho, Y. H. (2018). Drosophila melanogaster as a polymicrobial infection model for Pseudomonas aeruginosa and Staphylococcus aureus. *Journal of Microbiology, 56*(8), 534-541.

Liu, B., Zhang, J., and Guo, H. (2022). Research progress of polyvinyl alcohol water-resistant film materials. *Membranes, 12*(3), 347.

Liu, H., Wang, C., Li, C., Qin, Y., Wang, Z., Yang, F., Li, Z., and Wang, J. (2018). A functional chitosan-based hydrogel as a wound dressing and drug delivery system in the treatment of wound healing. *RSC Advances, 8*(14), 7533-7549.

Ma, Q., Du, L., Yang, Y., and Wang, L. (2017). Rheology of film-forming solutions and physical properties of tara gum film reinforced with polyvinyl alcohol (PVA). *Food Hydrocolloids, 63*, 677-684.

Malone, M., and Schultz, G. (2022). Challenges in the diagnosis and management of wound infection. *British Journal of Dermatology, 187*(2), 159-166.

Matica, M. A, Aachmann, F. L., Tøndervik, A., Sletta, H., and Ostafe, V. (2019). Chitosan as a wound dressing starting material: Antimicrobial properties and mode of action. *International Journal of Molecular Sciences, 20*(29), 5889.

Mori, N. M, Patel, P., Sheth, N. R., Rathod, L. V., and Ashara, K. C. (2017). Fabrication and characterization of film-forming voriconazole transdermal spray for the treatment of fungal infection. *Bulletin of Faculty of Pharmacy Cairo University, 55*(1), 41-51.

Mothilal, N. P., Pradeep, A., Arthi, C., Gopal, K., Kaliannagounder, V. K., Park, C. H., Kumar, V. A., and Rangasamy, J. (2024). Amikacin sulphate loaded chitosan-diopside nanoparticles composite scaffold for infectious wound healing. *International Journal of Biological Macromolecules, 263*, 130217.

Mulcahy, H., Sibley, C. D., Surette, M. G., and Lewenza, S. (2011). Drosophila melanogaster as an animal model for the study of Pseudomonas aeruginosa biofilm infections in vivo. *PLoS pathogens, 7*(10), 1002299.

Mulder, G. D., Cavorsi, J. P., and Lee, D. K. (2007). Polyhexamethylene Biguanide (PHMB): An addendum to current topical antimicrobials. *Wounds, 19*(7), 173-182.

Nandiyanto, A. B. D. (2026). How to read and interpret FTIR spectra for materials: A master dataset with step-by-step guided peak-correlation analysis, representative examples, and a foundation for future artificial intelligence (AI)-assisted analysis. *ASEAN Journal for Science and Engineering in Materials*, *5*(2), 323-356.

Negut, I., Grumezescu, V., Grumezescu, A. M. (2018). Treatment strategies for infected wounds. *Molecules, 23*(9), 2392.

Nimal, T. R., Baranwal, G., Bavya, M. C., Biswas, R., and Jayakumar, R. (2016). Anti-staphylococcal activity of injectable nano tigecycline/chitosan-PRP composite hydrogel using Drosophila melanogaster model for infectious wounds. A*CS Applied Materials and Interfaces*, *8*(34), 22074-22083.

Oun, A. A, Shin, G. H, Rhim, J. W., and Kim, J. T. (2022). Recent advances in polyvinyl alcohol-based composite films and their applications in food packaging. *Food Package Shelf Life, 34*, 100991.

Pandian, M., Selvaprithviraj, V., Pradeep, A., and Rangasamy, J. (2021). In-situ silver nanoparticles incorporated N, O-carboxymethyl chitosan based adhesive, self-healing, conductive, antibacterial, and anti-biofilm hydrogel. *International Journal of Biological Macromolecules, 188*, 501-511.

Patrulea, V., Ostafe, V., Borchard, G., and Jordan, O. (2015). Chitosan as a starting material for wound healing applications. *European Journal of Pharmaceutics and Biopharmaceutics*, *97*, 417-426.

Rahman Khan, M. M., Rumon, M. M. H., and Islam, M. (2024). Synthesis, rheology, morphology, and mechanical properties of biodegradable PVA-based composite films: A review on recent progress. *Processes, 12*(12), 2880.

Sen, C. K., Roy, S., Mathew-Steiner, S. S., and Gordillo, G. M. (2021). Biofilm management in wound care. *Plastic and reconstructive surgery, 148*(2), 275e-288e.

Shetty, A., Dubey, A., Chrystle, J., Manohar, M., John, A., Amitha, N., Das, P., and Hebbar, S. (2024). Fabrication and in vitro characterization of curcumin film-forming topical spray: An integrated approach for enhanced patient comfort and efficacy. *F1000Research, 13*, 138.

Soujith, N. B. S., and Jawahar, N. (2023). Terbinafine HCl film-forming spray for the treatment of topical fungal infections. *Indian Journal of Pharmaceutical Education and Research*, *57*(1), 85-97.

Sowlati-Hashjin, S., Carbone, P., and Karttunen, M. (2020). Insights into the polyhexamethylene biguanide (PHMB) mechanism of action on bacterial membrane and DNA: A molecular dynamics study. *The Journal of Physical Chemistry B, 124*(22), 4487-4497.

Sritharadol, R., Nakpheng, T., Heng, P. W. S., and Srichana, T. (2017). Development of a topical mupirocin spray for antibacterial and wound-healing applications. *Drug Development and Industrial Pharmacy, 43*(10), 1715-1728.

Umar, A. K, Butarbutar, M., Sriwidodo, S., and Wathoni, N. (2020). Film-forming sprays for topical drug delivery. *Drug Design, Development and Therapy, 14*, 2909-2925.

Umar, A. K., Sriwidodo, S., Maksum, I. P., and Wathoni, N. (2021). Film-forming spray of water-soluble chitosan containing liposome-coated human epidermal growth factor for wound healing. *Molecules, 26*(17), 5326.

Wang, S., Ren, J., Li, W., Sun, R., and Liu, S. (2014). Properties of polyvinyl alcohol/xylan composite films with citric acid. *Carbohydrate Polymers, 103*, 94-99.

Wang, W. Y., Hu, H. W., Chiou, J. C., Yung, K. F., and Kan, C. W. (2023). Poly (hexamethylene biguanide) hydrochloride (PHMB)-based materials: synthesis, modification, properties, determination, and application. *Polymer Chemistry 14*(48), 5226-5252.

Wilkins, R. G., and Unverdorben, M. (2013). Wound cleaning and wound healing: a concise review. *Advances in Skin and Wound Care, 26*(4), 160-163.

Yang, E., Qin, X., and Wang, S. (2008). Electrospun crosslinked polyvinyl alcohol membrane. *Materials Letters, 62*(20), 3555-3557.