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How Substantial is the Effect of Polyhexamethylene Biguanide (PHMB) Irrigation Solution on a Wound's Biofilm?

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Abstract:
Background: Biofilm as a key component that contributes to the development of chronic wounds can increase the resistance of bacteria to antiseptic solutions. The current standard procedure for wound care involves the use of wound cleansing solutions such as povidone-iodine, chlorhexidine, and normal saline. Each of these solutions has limitations. One of the newer wound cleansing solutions is Polyhexamethylene Biguanide (PHMB). PHMB is known to inhibit the formation and disrupt the structure of biofilm. This study aims to conduct a systematic review and meta-analysis to evaluate the effectiveness of using PHMB as a wound irrigation solution in reducing biofilm in wounds. Methods: PubMed, Mendeley, ScienceDirect, and Cochrane were used as a database using the keywords for the analysis . Further exclusion using PICO criteria left us with 5 studies that met the inclusion criteria. Results: Our findings demonstrated a significant improvement in irrigation with PHMB group compared to the control group (MD= -5.09, 95% CI -8.40 to -1.78, p= 00001, I2= 99%,), there was significant biofilm reduction with the PHMB group compared to the control group (RD= 0.74, 95% CI 0.56 to 0.91, p= 00001, I2= 0%). Conclusion: Compared to standard wound irrigation solutions, PHMB presents several advantages. It acts as a potent bactericidal agent against biofilm-forming microbes, including both Gram-positive (Staphylococcus aureus and MRSA) and Gram-negative bacteria (Pseudomonas aeruginosa). Additionally, PHMB is effective against fungi (Candida albicans), does not induce pain, and is non-toxic to healthy cells and granulation tissue.

Keywords: *Polyhexamethylene Biguanide, Biofilm, Wound, Wound care, Irrigation solution.*

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Introduction

Biofilms are a complex community of microorganisms, primarily bacteria and fungi, that can impede wound healing and contribute to the development of chronic wounds. These microbial communities may adhere to the wound surface or exist as aggregates without direct attachment to the surface (Roy et al., 2017). Biofilms can offer numerous advantages to microorganisms, including safeguarding them from the immune system and antimicrobial/antibiotic agents, shielding them from desiccation, providing storage for nutrients, and amplifying extracellular enzymatic activity, all of which contribute to the difficulty in eradicating the microbes within (Zhao et al., 2023). Typically, wound irrigation in clinical or hospital settings utilizes solutions such as povidone-iodine, chlorhexidine, and normal saline (Lewis & Pay, 2024). These solutions exhibit several limitations. For instance, povidone-iodine exhibits mild toxicity towards healthy cells and granulation tissue, potentially causing discomfort.

Similarly, chlorhexidine demonstrates reduced bactericidal efficacy against Gram-negative bacteria. Furthermore, normal saline lacks inherent antiseptic properties (Lewis & Pay, 2024). Topical irrigation of wounds with antimicrobial or anti-biofilm solutions can facilitate biofilm disruption and promote accelerated wound healing (Haapasalo & Shen, 2010).

The application of Polyhexamethylene Biguanide (PHMB) solution as a wound irrigation fluid can help suppress the establishment of new biofilm and disrupt the structure of previously formed biofilm, thereby facilitating a more expeditious wound healing process and mitigating biofilm-associated complications (Zheng et al., 2021). PHMB, a positively charged cation, is capable of interacting with the negatively charged surfaces of bacteria and biofilms.

Due to its cationic nature, PHMB can form electrostatic bonds with the negatively charged surfaces of microbes and biofilms. This interaction can lead to disruption of the biofilm structure, thereby inhibiting the formation of new biofilms and dismantling the structure of existing ones (Wei et al., 2009). The aim of this systematic review and meta-analysis is to evaluate the efficacy of PHMB irrigation solution in diminishing biofilm on wounds and promoting the wound healing process, as observed through the physical quantification of bacteria and biofilm using microscopic examination.

This study aims to analyze the extent of the influence of Polyhexamethylene Biguanide (PHMB) irrigation solution on the biofilm on wounds, as well as its impact on the healing process of infected wounds. The results of the study are expected to provide a deeper understanding of the effectiveness of PHMB in reducing or destroying biofilms, as well as assessing the potential of PHMB as an alternative treatment for hard-to-heal infectious wounds. The benefits of this study include contributing to the development of more effective wound therapies, providing a scientific basis for the use of PHMB in the treatment of infectious wounds, and providing guidance for further research and medical practice in treating wound biofilm infections.

Methods

The systematic review was registered in PROSPERO (CRD42024595275). Data search through various databases, such as PubMed, Mendeley, ScienceDirect, and Cochrane, using the keyword combinations "Polyhexamethylene Biguanide", "PHMB", "biofilm", "wound", "anti-biofilm". The search was conducted until October 12, 2024.

This study employed the PICO framework as the inclusion criteria. The population encompassed various types of biofilm models and microorganisms responsible for biofilm formation, including Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, and Candida albicans. The intervention investigated was the use of PHMB wound irrigation solution to reduce biofilm on wounds. The comparisons examined involved the utilization of other wound irrigation solutions, such as physiological saline solution, or treatment without wound irrigation solution. The outcomes measured included the number or percentage of bacteria in log CFU/g units, as well as the physical observation of biofilm structure using a microscope after the administration of PHMB wound irrigation fluid. The study designs ranged from in vitro, ex vivo, to in vivo. The exclusion criteria for this study were studies that did not assess the results of PHMB irrigation on biofilm, articles not published in English, case reports, literature reviews, studies unable to access the full paper, and those lacking critical information.

Four researchers independently reviewed and screened articles from the predefined database. Data extraction was carried out independently by four reviewers, who also evaluated the quality of the studies using a risk of bias assessment tool. The extracted data encompasses the authors' names, study design, interventions, and results.

The researchers conducted a meta-analysis to integrate and summarize quantitative and qualitative data from multiple studies. They utilized the Revman 5.4 software to report Mean Differences and 95% Confidence Intervals for continuous outcomes, as well as Risk Difference and 95% Confidence Intervals for dichotomous variables. To assess heterogeneity across trials, the Chi-square test was employed, while the I2 statistic was used to measure inconsistency. When I2 was less than 50% and p-value exceeded 0.1, a fixed-effects model was employed to pool the data. In contrast, a random-effects model was utilized for data analysis when these conditions were not met.

Results and Discussion

Search and Selection of Studies

A total of 46 potential studies were initially identified, and following a thorough review, 26 studies were obtained. Ultimately, 5 studies were determined to meet the inclusion criteria. The process of identifying these studies is visually represented in Figure 1.

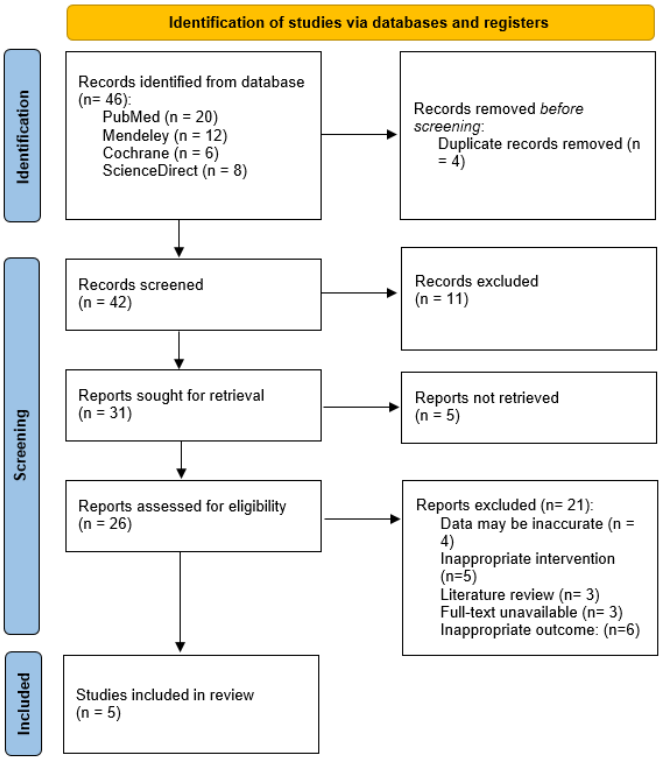


Figure 1. The process of identifying studies.

Characteristics of Included Studies

The studies examined in this review are summarized in Table 1. The published dates ranged from 2017 to 2023, with a total of 227 specimens utilized for bacterial enumeration and 68 specimens for microscopic biofilm analysis. The bacterial species investigated included Pseudomonas aeruginosa, Staphylococcus aureus, MRSA, Enterococcus faecalis, and Candida

albicans. The microscopic techniques employed were Confocal Microscopy, Epifluorescent Microscopy, and Laser Scanning Microscopy.

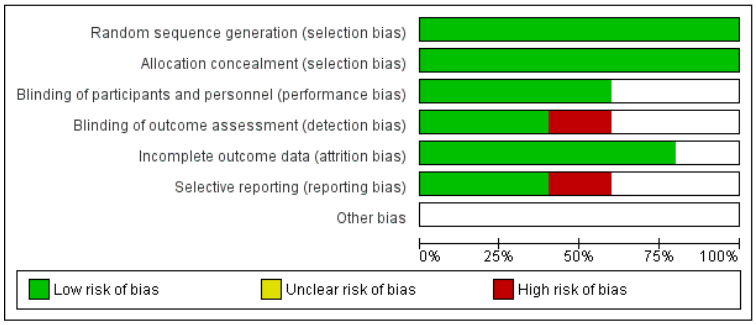
Tabel 1. Characteristics of studies.

Author (Year)	Study Design	Population and Sample	Intervention	Result (and significance p value)
Davis et al. (2017)	Ex Vivo	Porcine Wound Model Biofilm	Administration of PHMB, Ringer's solution, HOCl, sterile water, Octenidine dihydrochloride, and octenilin.	The PHMB treatment group demonstrated the most substantial decrease in bacterial burden compared to the other study cohorts. Specifically, the bacterial counts on days 3 and 6 in the PHMB group were 5.75±0.47 log CFU/g and 4.34±0.67 log CFU/g, respectively, in contrast to the control group's count of 7.42±0.49 log CFU/g.
Dittmer et al. (2023)	In Vitro	lhBIOM (Leucocyte-Rich Human-Plasma Biofilm Model	Administration of an irrigation solution containing 0.1% octenidine dihydrochloride and 0.1% PHMB for 24, 48, and 72 hours	Treatment with PHMB resulted in a significant decrease ($P<0.05$) in colony counts of the *P. aeruginosa* + *S. aureus* and *P. aeruginosa* + *E. faecium* co-culture groups at 24, 48, and 72 hours compared to the untreated control.
Paleczn et al (2023)	In vitro	Bacterial (*Staphylococcus aureus* and *Pseudomonas aeruginosa*) and Fungi (*Candida albicans*) Biofilm	Compare the efficacy of low hypochlorite-containing solutions vs. polyhexanide-containing antiseptic	PHMB demonstrated an 80% biofilm eradication rate against S. aureus and C. albicans, and a 70% rate against P. aeruginosa in the CBB model ($P<0.05$).
Gryson et al (2023)	In vitro	Bacterial (*Pseudomonas aeruginosa, Staphylococcus aureus, Enterococcus faecalis*, MRSA) and fungal (*Candida albicans*) Biofilm	10% Povidone-Iodine (PVP-I), 0.1% Polyhexamethylene Biguanide (PHMB), kontrol Phosphate-buffered saline (PBS)	PHMB is as effective as PVP-I ($P<0.05$), but slower acting.
Krasowski et al (2022)	Confocal and epifluorescent microscopy analysis (In vitro)	*Staphylococcus aureus* Biofilm	Polyhexanide (PHMB), Povidone–Iodine (PVP-I), dan Sodium Hypochlorite (NaOCl), assessed through Biofilm-Covered Area (BCA) and Biofilm Fluorescence Intensity Drop (BFID)	Significant biofilm eradication for PHMB and PVP-I ($p< 0.05$)

Risk of Bias Included Studies

The methodological quality of the five included studies is shown in Figure 2. Most studies of randomization have a low risk of bias.

A.



B.

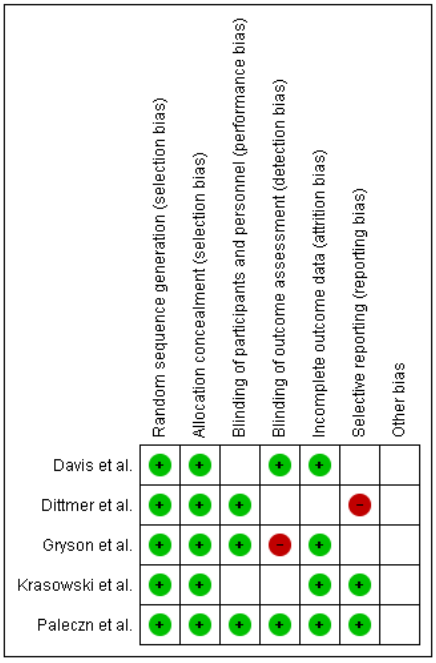


Figure 1. Risk of bias assessment. (A) Risk of bias graph. (B) Risk of bias summary.

1. Analyses of Outcome

1) Bacterial Count

Our findings demonstrated a significant improvement in irrigation with PHMB group compared to the control group (MD= -5.09, 95% CI -8.40 to -1.78, p= 00001, I2= 99%. Our findings demonstrated a significant biofilm reduction with PHMB group compared to the control group (RD= 0.74, 95% CI 0.56 to 0.91, p= 00001, I2= 0%.

Discussion

The antimicrobial agent polyhexamethylene biguanide has garnered significant attention for its potential to effectively combat biofilm-related wound complications (Mendoza et al., 2019). According to the review of the study in Table 1, it was found that the use of PHMB can

significantly reduce the number of bacterial colonies in vitro and ex vivo on day 3 compared to the control. Studies conducted by Dittmer et al. (2023) and Johani et al. (2018) showed that the use of PHMB observed for only 24 hours resulted in a significant reduction in the number of bacteria studied (Dittmer et al., 2023; Johani et al., 2018). The study conducted by McMahon et al. (2020) employed PHMB on MRSA, Pseudomonas aeruginosa, and Candida albicans specimens and observed them for 15 minutes. This shorter time frame yielded a notable reduction in all three microbial populations (McMahon et al., 2020). Numerous studies have demonstrated that PHMB, even at relatively low concentrations as low as 0.02%, exhibits a strong and potent inhibitory effect on the growth, initial attachment, and overall formation of biofilms produced by various pathogens commonly found in hospital-acquired infections (Chindera et al., 2016).

These pathogens include a wide range of Gram-negative bacteria, Gram-positive bacteria, and even pathogenic fungi (Donlan, 2002). Furthermore, research has shown that PHMB has the remarkable ability to disrupt and eradicate pre-formed biofilms of these problematic microorganisms (Zheng et al., 2021). This dual capability of both preventing biofilm formation and dismantling existing biofilms suggests that PHMB-based wound cleansers hold great potential as an effective strategy in the treatment and management of both acute and chronic infected wounds that are complicated by the presence of resilient biofilm formations.

The standard fluids employed in wound care procedures include povidone-iodine, chlorhexidine, and normal saline (Lewis & Pay, 2024). Povidone-iodine has demonstrated a wide-ranging bactericidal capacity, exhibiting efficacy against both Staphylococcus aureus and Pseudomonas species, in addition to an inhibitory effect on biofilm formation (Hoekstra et al., 2017). However, povidone-iodine demonstrates cytotoxic effects on healthy cells and granulation tissue and may elicit discomfort if not adequately diluted (Lewis & Pay, 2024). Chlorhexidine demonstrates a potent bactericidal activity against Gram-positive bacteria and fungi, yet its efficacy is reduced against Gram-negative bacteria (Karpi & Szkaradkiewicz, n.d.). Moreover, chlorhexidine demonstrates toxicity to the corneal epithelium, even at low concentrations, rendering it unsuitable for use in the ocular area (Bever et al., 2016). Meanwhile, normal saline is not an antimicrobial agent and does not effectively disrupt biofilm formation (Davis et al., 2017; Lewis & Pay, 2024).

The mechanism of PHMB's antimicrobial action is believed to involve a multifaceted approach that disrupts the microbial cell membrane and interferes with cellular processes. The positively charged PHMB molecules are attracted to the negatively charged bacterial cell surface and insert into the lipid bilayer, causing membrane destabilization and leakage of cellular contents (Chindera et al., 2016). This membrane disruption leads to the loss of cellular homeostasis and the eventual death of the microbial cell. Additionally, PHMB has been shown to selectively condense bacterial chromosomes, impairing DNA replication and gene expression, and further contributing to the termination of the microbial life cycle (Chindera et al., 2016).

This dual mechanism of action, simultaneously targeting the cell membrane and the genetic material, is a key factor in PHMB's potent antimicrobial properties and its ability to

effectively kill a wide range of microorganisms, including both Gram-positive and Gram-negative bacteria, as well as pathogenic fungi. PHMB exhibits a strong inhibitory effect on the growth, initial attachment, and biofilm formation of both Gram-negative bacteria, such as Pseudomonas aeruginosa and Escherichia coli, and Gram-positive bacteria, such as Staphylococcus aureus, and pathogenic fungus, such as Candida albicans (Davis et al., 2017; Dittmer et al., 2023; Gryson et al., 2023; Krasowski et al., 2022; Paleczny et al., 2023).

Different studies might use varying concentrations of PHMB, different salt forms (e.g., hydrochloride, gluconate), or even different formulations with varying excipients. This variability could lead to discrepancies in the observed effects on bacterial count and biofilm structure. The effectiveness of PHMB can vary depending on the specific bacterial species and even between different strains of the same species. Some species are inherently more susceptible to PHMB than others, and the presence of resistance mechanisms can also influence the outcomes. The methods used to grow biofilms in the laboratory can significantly impact their structure and susceptibility to antimicrobials.

Factors like the type of surface used for biofilm growth, the nutrient composition of the medium, and the duration of biofilm development can all introduce variability. Different methods for quantifying bacteria, such as plate counting, flow cytometry, or qPCR, have varying levels of sensitivity and might not be directly comparable. This could lead to discrepancies in the reported bacterial counts between studies. The choice of microscopic techniques (e.g., confocal laser scanning microscopy, scanning electron microscopy) and the criteria used to assess biofilm structure can vary between studies, potentially leading to subjective interpretations of the results.

While PHMB is typically considered safe, it is important to be cognizant of potential side effects. PHMB has been known to induce mild cutaneous irritation in some users, particularly when applied at high concentrations up to 2% or with prolonged exposure. Manifestations of this skin irritation may encompass erythema, pruritus, or a burning sensation. Additionally, though infrequent, allergic responses to PHMB have been documented, with a spectrum of presentations ranging from dermatitis to anaphylaxis (Kolodziej et al., 2021; Sukakul et al., 2020).

Ongoing investigations are essential to understand the potential for bacteria to develop resistance to PHMB through continuous exposure, which can inform the development of long-term strategies for utilizing PHMB as an anti-biofilm agent. Future research should prioritize in vivo studies using animal models and clinical trials to validate the findings from in vitro experiments, as well as to evaluate the efficacy and safety of PHMB in clinical applications, such as the treatment of biofilm-related infections in humans. Research demonstrating the effectiveness of PHMB against diverse bacterial types, including antibiotic-resistant strains, can support the adoption of PHMB as a preferred anti-biofilm agent in wound care guidelines. This research can be leveraged to educate healthcare professionals about the significance of biofilm management in wound care and the role of PHMB in this approach.

Conclusion

Compared to standard wound irrigation solutions, PHMB presents several advantages. It acts as a potent bactericidal agent against biofilm-forming microbes, including both Gram-positive (Staphylococcus aureus and MRSA) and Gram-negative bacteria (Pseudomonas aeruginosa). Additionally, PHMB is effective against fungi (Candida albicans), does not induce pain, and is non-toxic to healthy cells and granulation tissue.

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