THE SECRET OF ANTIBIOFILM AGENT FOR BURN INJURY : A SYSTEMATIC REVIEW

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Abstract

Burn injuries remain to be a significant issue. The environmental condition in Indonesia contributes to the prevalence of diverse bacterial infection associated with different types of wounds, including burns. The biofilms production at the site of burn wounds significantly contributes to the ineffectiveness of burn treatment protocols.
Hereby in this paper we discuss about the mechanism of antibiofilm and the novel therapy for the burn injury.
This study was classified as a systematic review approach. The compilation of references will be derived from journal articles obtained using search engines such as PubMed, Google Scholar, and EBSCO. Subsequently, the authors proceed to gather and reevaluate pertinent material and scholarly articles.
The occurrence of biofilm is closely associated with burn injuries. These bacterial communities evoke antibiotic resistance, hence impeding the effectiveness of conventional treatment approaches. It is noteworthy that substantially 60% of burn-related mortality is attributed to biofilms. This condition has the potential to develop into a persistent wound, ultimately resulting in sepsis. The development of biofilm or invasive burn wound infection serves as a significant catalyst for the occurrence of sepsis. The administration of anti-biofilm drugs holds significant potential in this context as a proactive measure for reducing the worsening effects of burns.
The utilization of anti-biofilm agents has the potential to serve as an innovative therapeutic approach for the treatment of burn wounds in the future. Further investigation is required to explore the potential outcome as well as adverse effects of this drug linked to its potency.

Keywords : Biofilm formation; Burn treatment; Burn wound; Septicemia

INTRODUCTION

Burns injuries are substantially related with bacterial infection. The possibility of these bacterial infection are increases depends on burns conditions. Infection of burn injuries is common complication, and is a major cause of death in burn patient. More than 70% deaths occurs in burn patients due to infection and these patient remain at risk of mortality until unless burn wound does not healed completely. Infection in burns mostly infected and initially colognized by Gram positive bacteria, mainly Staphylococci are found during first post burn days followed by gram negative bacteria. The predominant bacteria isolated were *Staphylococcus aureus* (33.85%), followed by *Pseudomonas spp.* (18.46%), *Acinetobacter baumannii* (15.38%), *Klebsiella pneumonia* (13.85%), *Escherichia coli* (8.46%) and *Proteus mirabilis* (4.42%).[1] Furthermore, a research investigation examining the impact of DNase-I on microbial biofilm and wound healing efficacy revealed that the utilisation of a chitosan gel containing solid lipid nanoparticles of silver sulfadiazine (SSD-SLNs) and supplemented with DNase-I demonstrated a successful therapeutic approach for addressing biofilm-related wound infections.[2,3] Antibiofilm compounds, which exhibit the ability to inhibit the formation of biofilms, are employed not only as therapeutic interventions but also demonstrate enhanced efficacy as primary pharmaceutical treatments for burn injuries. The primary objective of implementing antibiofilm agents in wound care is to mitigate the risk of infection, enhance the efficacy of antibiotics, expedite the wound healing process, and minimise the likelihood of resistance development in the skin. It is imperative to acknowledge that the aforementioned remedies are currently under investigation and may not have attained widespread accessibility at present. The antibiofilm mechanism of SSD remains unclear and needs to investigate further. Others agent that contains antibiofilm mechanism were suggested to be treatment in burn injury. Hereby in this paper we discuss about the mechanism of antibiofilm and the novel therapy for the burn injury.

METHODS

The selected methodology entails conducting an systematic review. The process involves doing a comprehensive review of relevant scholarly literature sources pertaining to the correlation between the antibiofilm and burn injury. The data collection process involved the utilisation of secondary data obtained from various search engines, including PubMed, ProQuest, and Cambridge Core. Following the data collection process, the gathered data is next submitted to thorough examination and subsequently undergoes various data reduction processes. Following this, the processed content is then presented and organised based on the journal from which it originated. Figure 1 displays the stages involved in doing a data search, which incorporates the PRISMA flow chart.

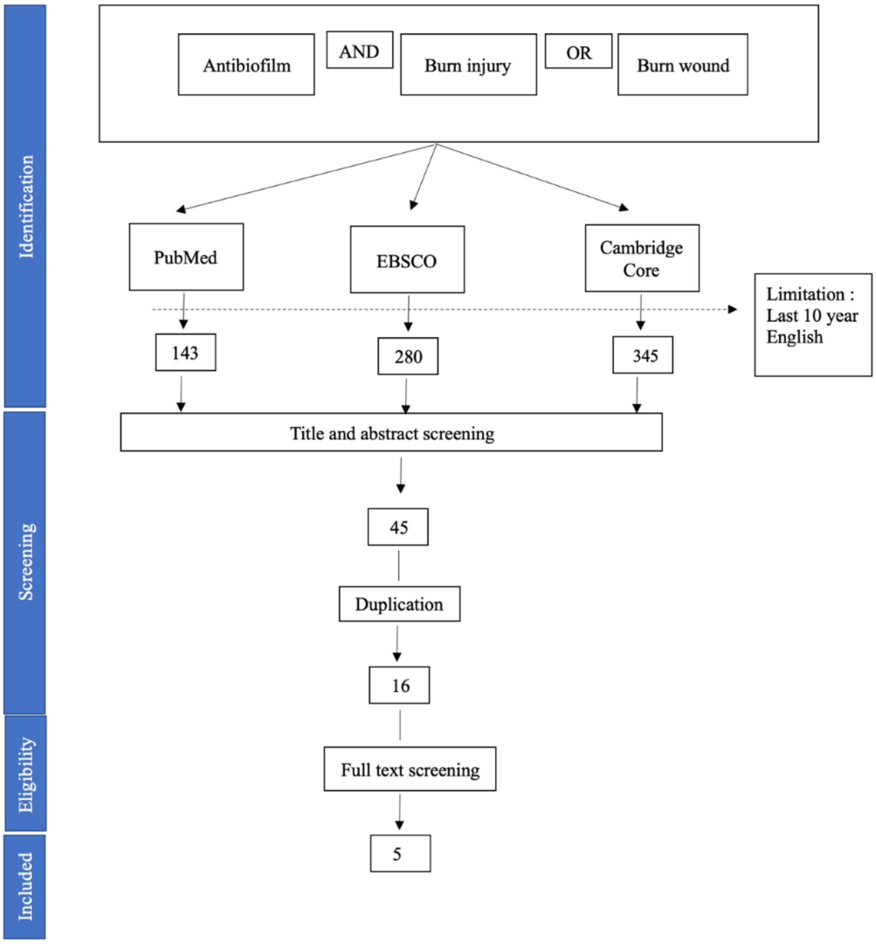


Figure 1. PRISMA Flowchart for studie

RESULT

The analysis of the five journals revealed the presence of diverse anti-biofilm activities that exhibit potential as novel therapeutic agents for the treatment of burn wounds. The information is presented in a concise manner in Table 1.

Table 1. Journal matrix of the studies

Studies	Journal Type	Quintessence	Supplementary Data
Song et al. 2016	Original research article	Chlorhexidine acetate nanoemulsion (CNE) demonstrates antimicrobial efficacy against *methicillin-resistant Staphylococcus aureus* (MRSA) in both laboratory settings (in vitro) and living organisms (in vivo) by facilitating the inhibition of biofilm development and aiding the elimination of existing biofilms.	The relative electrical conductivity and leakage of alkaline phosphates, K^+, $Mg^{2+,}$ DNA, and protein were seen to exhibit a clear rise, which can be attributed to the damage incurred by the cell wall and membrane.
Ma et al. 2017	Original research article	WRL3 exhibits significant efficacy against microorganisms involved in biofilm development.	MRSA has demonstrated notable effectiveness in vitro, as well as exhibiting a high level of success in the treatment of MRSA burn wound infections in vivo.
Pallavali et al. 2021	Original research article	The concept of utilising lytic bacteriophages as potential substitutes for antibiotics in the treatment of bacterial illnesses.	N/A
Pourhajibagher et al. 2021	Original research article	The effectiveness of sonodynamic therapy (SDT) utilising nano-emodin (N-EMO) in combating multi-species bacterial biofilms.	The gene expression profiling encompasses the analysis of abaI, agrA, and lasI genes have the potential to modulate the activity involved in the development of bacterial resistance.

Regulski et a. 2023	Original research article	The effectiveness of wound care products in inhibiting biofilm formation.	The BDWG product had the highest efficacy in reducing the formation of mature biofilms, with reductions above 2-log for both *S. aureus* (5.88) and *P. aeruginosa* (6.58).

Following acquiring a journal for subsequent analysis, the journal is subsequently examined utilising the PICO assessment tool. The selection of keywords is contingent upon the specific combinations of PICO elements employed. The scope of searches is restricted to the titles and abstracts of documents. Regarding instances where the number of retrieved papers exceeded 200, the search was restricted to encompass solely randomised controlled trials, experimental studies, systematic reviews, and meta-analyses. The investigation excluded articles that were either duplicated or retracted across databases, with the assessment being based on the similarity of their title names and content. Articles that were published more than 10 years ago were excluded from consideration, unless there was no systematic review available within the past decade. The process involved the selection of pertinent articles, followed by their consolidation and subsequent summarization. The researchers adhered to several standard search methods for conducting systematic reviews, but made modifications to provide a practical and succinct search approach. A comprehensive replication of a systematic review was not undertaken. This task entails achieving a harmonious equilibrium among the dimensions of breadth, relevancy, and the quantity of unrestricted textual expressions employed. Table 2 provides a comprehensive description of the PICO analysis.

Table 2. PICO Analysis for studies

Study	**Type of Study**	**Population**	**Intervention**	**Comparison**	**Outcome(s)**
Song et al. 2016	Experimental study (*in vitro* and *in vivo)*	MRSA (methicillin-resistant Staphylococcus aureus) from burn injury patient	Chlorhexidine acetate nanoemulsion (CNE)	water solution (CHX)	CNE exhibits considerable potential as an antibacterial agent, particularly in the context of treating MRSA infections in skin burn wounds.

Ma et al. 2017	Experimental study (*in vitro*)	Isolated MRSA from burn injury patient	Membrane-Active Amphipathic Peptide WRL3	N/A	The WRL3 has capacity proliferation of cells in wound tissue, minimising the presence of harmful microorganisms and promoting a conducive environment for the process of wound healing.
Pallavali et al. 2021	Experimental study(*in vitro*)	Cultured from burn injury patient	Antibiofilm based Lytic Bacteriophages	N/A	Antibiotics that use the pathophysiology of lytic bacteriophages have the potential to serve as a viable alternative to antibiofilm agents.
Pourhajibagher et al. 2021	Experimental study (*in vitro*)	Cultured from burn injury patient : *P. aeruginosa*, *S. aureus*, and *A. baumannii*	nano-emodin-mediated sonodynamic therapy (N-EMO)	N/A	Reducing the formation of biofilms in multi-species bacterial growth among patients with burn injuries.
Regulski et a. 2023	Experimental study (*in vitro*)	Isolated *Staphylococcus aureus* and *Pseudomonas aeruginosa* from burn injury patient	Wound care products	Untreated burn injury infection	Product containt Nano Ag and CMC-Cellulose 1.7% Ag, along with the Collagenase product and the antimicrobial gel-BDWG, has significant efficacy in inhibiting the production of new biofilms by both *S. aureus* and *P. aeruginosa*.

Subsequently, the evaluation of bias risk is conducted by the use of instruments that encompass a series of guiding inquiries. Figure 2 presents an overview of the various causes of bias associated with each journal and the corresponding research acquired. Based on the acquired data, it is evident that all journals included in the study conducted experimental research. Consequently, in order to evaluate the risk of bias, an assessment instrument derived from the work of Tufanaru et al. (2020) was employed.[4]

Table 3. Risk of Bias in experimental studies (Adapted from Tufanaru et al. 2020)

Risk of Bias question	Song et al. 2016	Ma et al. 2017	Pallavali et al. 2021	Pourhajibagher et al. 2021	Regulski et a. 2023
Is it clear in the study what is the ‘cause’ and what is the ‘effect’ ?	🟢	🟢	🟢	🟢	🟢
Were the participants included in any comparisons similar ?	🟡	🟡	🟡	🟡	🟢
Were the participants included in any comparisons receiving similar treatment/care, other than exposure of intervention of interest ?	🟢	🟢	🟢	🟢	🟢
Was there a control group ?	🟡	🔴	🔴	🔴	🟢
Were there multiple measurements of the outcome both pre and post the intervention ?	🟡	🟡	🟡	🟡	🟡
Was follow up complete, and if not, were the differences between group in terms of their follow up adequately described and	🟢	🟢	🟢	🟢	🟢

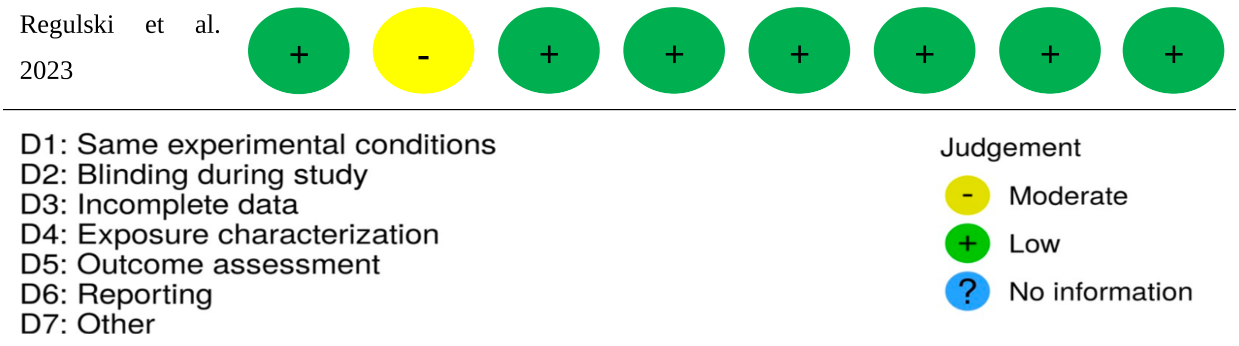
analyzed ?					
Were the outcomes of participants included in any comparisons measured in the same way ?					
Were outcomes measured in a reliable way ?					
Was appropriate statistical anaylsis used ?					

Low risk of bias Unclear risk of bias High risk of bias

The assessment of bias techniques included in Table 4 are utilised to compare the design and output of many research, with the aim of ascertaining the suitability of the data for usage and identifying any potential risks of influencing subsequent conclusions. The utilisation of these instruments is classified for in vitro investigations. The inclusion criteria for this systematic review were based on the nature of the investigations, which were only in vitro studies. Therefore, these specific parameters were chosen for the analysis.

Table 4. The assessment of bias in in vitro investigations. The study utilised the risk-of-bias technique created by the United States National Toxicology Programme to assess in vitro investigations.

	D1	D2	D3	D4	D5	D6	D7	Overall
Song et al. 2016	+	-	+	+	+	+	+	+
Ma et al. 2017	+	-	+	+	+	+	+	+
Pallavali et al. 2021	+	-	+	+	+	+	+	+
Pourhajibagher et al. 2021	+	-	+	+	+	+	+	+



DISCUSSION

A biofilm is an organised cluster of microorganisms who have attached to a surface, whether alive or not alive, then have gotten united in a group. The mechanism of biofilm formation begins by the initial adhesion of cells.[3] Burn wounds commonly develop polymicrobial biofilms. However, the majority of persistent wound biofilms consist of multiple types of microorganisms, with S. aureus and P. aeruginosa frequently being the most common species. Bacterial communities consisting of multiple species, surrounded by a material called extracellular polymeric substance (EPS), has intrinsic resistance to antibiotics, antiseptics, and antimicrobials, leading in the development of antibiotic resistance. The challenge of antibiotic drug resistance is exacerbated by the accelerated rate at and these resistance develops in the microbial community. Biofilms provide a serious threat in the setting of burns, with approximately 60% of burn-related mortality related to the presence and impact of biofilms. The adaptability of biofilms among bacteria is a key factor promoting to antibiotic resistance, as well as exacerbating complications associated with burns. Moreover, these present a considerable obstacle in the context of persistent wounds, such as those linked to diabetes, hypertension, and venous leg ulcers.[5] Afterwards, cells progress to the irreversible adhesion phase and initiate the formation of the extracellular matrix (ECM). The extracellular matrix (ECM) consists predominantly of water and is sustained by many components such as polysaccharides, proteins, lipids, and extracellular DNA (eDNA), which contribute for its structural and functional integrity. After the cellular encapsulation is initiated in the extracellular matrix, the biofilm reaches the maturation phase. The bacteria present in a mature biofilm possess the capability to distribute resources through channels and exhibit response to both internal and external environmental stimuli. Particulates and planktonic cells are separated from the extracellular matrix during the dispersal phase, which marks the conclusion of the biofilm maturation process. These dispersed microorganisms subsequently develop colonies in new areas within the wound, so defining a repetitive cycle.[6,7] The process of biofilm generation can be elucidated through the visual representation provided in Figure 4

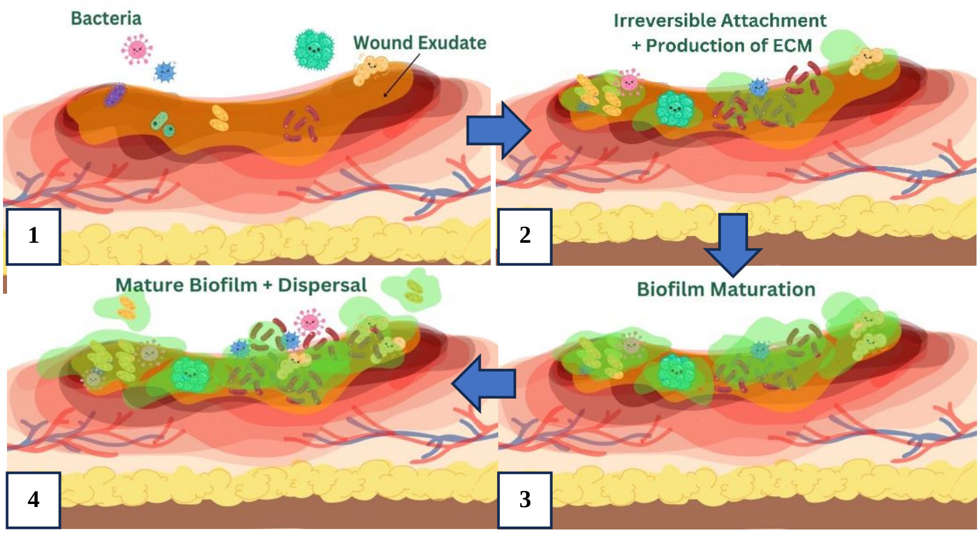


Figure 2. Biofilm formation (The image source was originally produced by the author)

A skin burn lesion facilitates the entry of germs present in the skin tissue, leading to systemic processes and septic organ failure. During colonisation of wounds by biofilms, there occurs a significant increase in morbidity and death among patients with skin wounds, as well as an escalation in treatment costs.[8] Hospital-acquired infections have been predominantly initiated by a range of antibiotic-resistant bacteria, such as *vancomycin-resistant enterococcus* (VRE), MRSA, *Klebsiella pneumoniae*, *multidrug-resistant Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and bacteria that generate *extended spectrum beta-lactamases* (ESBL).[9] Mixed-species biofilms are common in wounds, including burns, approaching single-species biofilms in incidence. Among them, *S. aureus* and *P. aeruginosa* are the most often identified species.[10] Burn wounds or burn injuries result in significant skin damage, making the patient more vulnerable to bacterial infections, especially those caused by bacterial biofilms.

Burns rapidly become colonised by Gram-positive bacteria coming from the patients' skin and contaminated surfaces in their immediate environment that patients come into contact with. Subsequently, within a timeframe ranging from hours to a few days, the wound gets inhabited by Gram-negative bacteria. A study conducted on burns in intensive care units (ICUs) and burn wards discovered the existence of 1621 bacteria in 2395 clinical samples. Out of these, 74.2% were categorised as Gram-negative bacteria, whilst 34.3% were specifically identified as *A. baumanii*. Bacterial contamination of burn sites often results in systemic sepsis and severe septicemia, leading to a substantial increase in the death rate among patients suffering from burn injuries. Infections are responsible for around 70% of mortality caused by burn injuries. Patients in this state are at risk of mortality until the burn wound has completely healed. The presence of immune cells in the affected area leads to a prolonged inflammatory response, which hinders the process of re-epithelization and wound closure. According to a meta-analysis conducted on the available literature pertaining to chronic wounds, it has been found that around 80% of these wounds are linked to the presence of a biofilm.[11] The precise significance of biofilm formation in the context of acute wounds remains uncertain and has been a subject of considerable scholarly discourse. One of the factors contributing to this ongoing discussion is the insufficiency of viable diagnostic methods for detecting the existence of biofilm in wounds.[12] Biofilms, however observed in acute wounds, occur at a relatively low occurrence rate of 6%. In vivo studies provide evidence that biofilms can develop in acute wound models as early as 3 days after trauma. Indeed, previous studies have shown that biofilm formation at the burn eschar might occur before the onset of systemic infection in in vivo models.[13] Currently, there is a worldwide need for medical intervention to treat severe skin burn injuries, with around 11 million persons needing treatment each year. Regrettably, this illness results in the death of 300,000 individuals annually.

Burn wound infection (BWI) is the predominant factor leading to sickness and mortality worldwide.

The predominant species identified at BWI include *S. aureus*, *P. aeruginosa*, and *Acinetobacter baumannii.* Recent research has confirmed the presence of multi-species biofilms composed of harmful bacteria in burn sites. Managing these infections poses a formidable challenge and, in some cases, proves to be ineffective. Therefore, it is imperative to provide proper treatment for burn wounds in order to reduce the likelihood of infection and improve the overall prognosis for the patient. Prompt and timely treatment of burns is crucial in order to prevent the colonisation of numerous bacteria, specifically *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. The analysis of tests on antimicrobial efficacy against biofilms revealed that there are five crucial model parameters that affect the results. These parameters include the ratio of biofilm surface area to volume, the density of cells in the biofilm, the conditions of fluid flow or static state over the biofilm, the age of the biofilm, and the choice of antibiotic used for comparison. The user's text is not clear and does not provide enough information to be rewritten. The current method of managing BWI entails a mix of wound debridement, superficial excision of necrotic tissue, and the application of antimicrobial medications either topically or systemically.[8]

Excessive use of antimicrobial drugs, particularly antibiotics, will result in an increased prevalence of microbial resistance, despite them being the main treatment choice. Therefore, it is essential to develop an innovative strategy to counteract the increase of antimicrobial-resistant strains and impede the formation of microbial biofilms.

The combination of antibiotics with bacterial resistance-modifying medicines, which enhance the efficacy of the antibiotic, has been acknowledged as a promising approach. In order to prevent infection in burns, it is necessary to utilise topical medications that have antibacterial properties, such as oral antibiotics and silver sulfadiazine. For several decades, silver sulfadiazine has been the primary choice for topical therapy. The medicine containing silver is used to treat burn wounds by absorbing the exudates and eliminating microorganisms that are attracted to the dressings. It binds to negatively charged proteins, RNA, and DNA, and destroys the cell walls of bacteria. Additionally, it prevents their reproduction, and reduces their metabolism and growth. Gholamrezazadeh observed that the presence of a nanomolecule composed of silver (nano-Ag) at a concentration of 12.5 ng/mL resulted in a decrease in the formation of biofilms by *P. aeruginosa* bacteria. However, a drawback of this approach is that partially and fully thickness burned areas have little permeability to these compounds. These products can also accelerate the development of drug-resistant types of pathogens.[8]

Based on the findings presented in Figure 1, it can be observed that a significant proportion of the questions assessed for risk of bias yielded outcomes indicating a low risk of bias. Nevertheless, it is imperative to underscore the significance of several periodicals. In relation to the matter of comparison, it is worth noting that just one study incorporated a comparison group.[10] The focus of this study is to evaluate the efficacy of anti-biofilm agents in wound care products. There exists a comparison group, specifically : Gauze , C. Nano Ag, extracellular poly- meric substance (EPS)-dissolving antimicrobial wound gel (BDWG), CMC-1.2% Ag , CMC- Cellulose- 1.7% Ag , G. Fish Skin , PU Poly-Sheet Foam-Ag Metallic , Salt Ag , J. Collagenas. The primary purpose of incorporating a comparison group inside risk of bias tools is to facilitate the evaluation of potential biases in a study by establishing a benchmark for comparison. The comparison group is commonly comprised of participants who are subjected to an alternative intervention or no intervention whatsoever, and serves as a means to assess the impact of the intervention under investigation. The Cochrane risk of bias tool incorporates a specific domain dedicated to evaluating selection bias. This domain evaluates the adequacy of randomization and concealment in the allocation of individuals to the intervention and comparison groups. This practise aids in establishing comparability between groups and enables the attribution of outcome differences to the specific intervention under investigation, rather than being influenced by extraneous factors. It is imperative to acknowledge that there is no singular, universally applicable instrument that comprehensively addresses the diverse range of settings in which the assessment of risk of bias is conducted. Hence, it is imperative to thoroughly deliberate upon the study design and context before choosing a tool or methodology for evaluating the potential for bias in systematic reviews.

The inclusion of a control group is a crucial consideration when assessing the potential bias of evaluation tools. A control group refers to a cohort of individuals participating in a research project who do not receive the experimental intervention under investigation. The primary objective of incorporating a control group in research studies is to establish a reference point for comparative analysis with the group that undergoes the intervention. This enables researchers to discern whether any observed effects can be attributed to the intervention itself or are influenced by extraneous variables.

According to the research conducted by Ma et al. (2017), it has been found that the proliferation of biofilm formation is regulated by the activity of Membrane-Active Amphipathic Peptide WRL3. The absence of a control group in this investigation can be attributed to the exclusive utilisation of MRSA samples derived from individuals afflicted with burn wounds.[8] In a similar vein, the study conducted by Pallavali et al. (2021) elucidated the efficacy of lytic bacteriophages in the antibiofilm mechanism exhibited by a cohort of multidrug-resistant bacteria, namely *Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae,* and *Staphylococcus aureus.* It is worth noting that the study did not include a control group.[14] Similarly, Pourhajibagher et al. (2021) elucidate the function of N-EMO in mitigating the formation of biofilms through SDT, albeit without the inclusion of a control group.[15] Furthermore, a control group is an essential component of a true experiment to increase the internal validity of the results and the confidence we can have in the conclusions. However, there are some instances when a control group is not included in an experimental design, such as when using a within-subjects design or a prospective cohort design.

The practise of assessing outcomes before and after an intervention is valuable for addressing and mitigating the influence of confounding variables and differential improvements within a group Outcome measurements utilised in clinical practise are categorised into four distinct groups: self-report measures, performance-based measures, observer-reported measures, and clinician-reported measures. None of the studies analysed provided a comprehensive explanation of the pre and post processes employed before to and during the research intervention. In experimental investigations, the absence of measurements of the outcome both before and after the intervention can pose challenges in determining the impact of the intervention. Nevertheless, it remains feasible to present the findings of the investigation by delineating the implemented intervention and documenting any discernible alterations in the subjects. Selecting appropriate outcome measures is a crucial aspect of conducting a study. These measures should possess clear and precise definitions, as well as accuracy, in order to facilitate researchers' comprehension of the relationship between variables, evaluate the advantages of a novel intervention, and enhance the overall well-being of patients. One of the implications that may be inferred from the data presented in Figure 2 indicates as certain study groups may still exhibit potential causes of bias. Moreover, it might be argued that this potential can be deemed acceptable.

The evaluation of bias in in vitro investigations holds significant importance in enhancing the precision and dependability of the outcomes, reinforcing the evidence and suggestions, identifying the origins of bias, enhancing the quality of studies, and advancing the development of more effective risk-of-bias assessment methods. One of the identified components in the assessment was the lack of explicit explanation regarding blinding procedures in the respective journals. The absence of a comprehensive explanation on blinding in the clinical journal can be attributed to the lack of sufficient contextual information. Blinding is a crucial component of clinical trials, which pertains to the deliberate concealment of group assignment from one or more participants participating in a clinical research study.

The implementation of blinding in a clinical study serves the purpose of mitigating bias resulting from participants' awareness of the specific intervention or control therapy they are receiving. The lack of blinding in a study can add bias into the results through several mechanisms, which are contingent upon the specific individuals participating in the study who are not blinded. For instance, in the absence of blinding among participants, there is a potential for altered behaviour or biassed reporting of outcomes due to their awareness of the specific intervention they were assigned to. If the outcome assessors lack blinding, there is a possibility that their awareness of the intervention administered to the participants could impact their judgements. The imperative to acknowledge that achieving blinding may not always be feasible across all study designs. In the context of a free-living experiment, it is not feasible to implement a blind design for assessing dietary intakes. In summary, the implementation of blinding in clinical trials plays a crucial role in mitigating potential biases. The inclusion of information regarding blinding in a particular clinical journal would be contingent upon the study's contextual factors and the reporting guidelines of the journal.[16]

CONCLUSION

Regarding summary, the occurrence of biofilm formation on infected burn wounds poses a substantial challenge in the management of burn injuries. This pathological state results in a prolonged healing process and an elevated susceptibility to complications and mortality. The findings of our investigation provide evidence that the inhibition of biofilm formation can hinder the transition from the first bacterial adhesion to the subsequent establishment of microcolonies and mature biofilms. The application of topical anti-biofilm preparations in the early stages of burn injuries is anticipated to be beneficial in mitigating the development of diverse strains of antibiotic-resistant bacterial infections. These infections have the potential to induce the creation of biofilm, which subsequently exacerbates the severity of the wound.

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None

CONFLICT OF INTERESET

None

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