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Review Article

Biofilm formation in cutaneous wounds and its behavior in the face of interventions: an integrative review

Formação do biofilme em ferida cutânea e seu comportamento diante das intervenções: revisão integrativa

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ABSTRACT
Objective: to identify in the literature the biofilm formation and its behavior when faced with interventions in cutaneous wounds. **Methods:** an integrative review, carried out in the Cumulative Index to Nursing and Allied Health Literature, Latin American and Caribbean Health Sciences Literature, EMBASE, Scopus, The Cochrane Library Collaboration, MEDLINE/PubMed and Science Direct databases, without temporal delimitation. Nineteen studies were selected. The information was evaluated descriptively, comparing it with the pertinent findings. **Results**: the sample studies were published in English and included three types of biofilm research: two clinical, six *in vitro* and 11 *in vivo* (animal). Three themes were included: biofilm model creation (n=4), biofilm assessment (n=3), biofilm behavior before interventions for its management (n=12). **Conclusion:** the detrimental effects of biofilm on wound healing have been confirmed. Several interventions were able to reduce and eliminate biofilm in *in vitro* and *in vivo* models. **Contributions to practice:** it was found that clinical evaluation of the lesion does not allow the identification of biofilm, even when present; it is below the surface of the lesion. This finding raises reflection on the part of nurses regarding the interventions adopted for the removal of biofilm.
Descriptors: Biofilms; Wounds and Injuries; Nursing; Enterostomal Therapy.

RESUMO
Objetivo: identificar na literatura a formação do biofilme e o seu comportamento diante das intervenções em feridas cutâneas. **Métodos:** revisão integrativa, realizada nas bases de dados *Cumulative Index to Nursing and Allied Health Literature*, Literatura Latino-Americana e do Caribe em Ciências da Saúde, EMBASE, Scopus, *The Cochrane Library Collaboration*, MEDLINE/PubMed e *Science Direct,* sem delimitação temporal. Foram selecionados 19 estudos. Avaliação das informações ocorreu de forma descritiva, confrontando com os achados pertinentes. **Resultados**: os estudos da amostra foram publicados no idioma inglês e contemplaram três tipos de pesquisa de biofilme: dois clínicos, seis in vitro e 11 in vivo (animal). Incluíram-se três temas: criação de modelo biofilme (n=4), avaliação do biofilme (n=3), comportamento do biofilme diante de intervenções para o seu manejo (n=12). **Conclusão:** efeitos prejudiciais do biofilme na cicatrização de feridas foram confirmados. Diversas intervenções foram capazes de reduzir e eliminar o biofilme nos modelos *in vitro* e *in vivo*. **Contribuições para a prática:** constatou-se que avaliação clínica da lesão não permite identificar o biofilme, inclusive quando presente encontra-se abaixo da superfície da lesão. Este achado suscita reflexão por parte dos enfermeiros a respeito das intervenções adotadas para a remoção do biofilme.
Descritores: Biofilmes; Ferimentos e Lesões; Enfermagem; Estomaterapia.

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Introduction

The discussion regarding the influence of biofilm in the delay of wound healing has become frequent in the 21st century. Chronic wounds of various etiologies that do not progress to healing, despite comprehensive patient evaluation and adoption of specific interventions to remedy or mitigate the problems, are suspected to have biofilm[1].

Biofilms are defined by microorganisms attached to each other or to a surface, enclosed in a matrix of extracellular polymeric substance, forming a mechanism for resistance and survival[2]. They are present in chronic wounds, being found on the injured surface, suspended in the exudate, adhered to necrotic tissue or in the structure of the dressings, usually called "bandages"[3].

The process of wound tissue repair is by mechanisms not fully understood. Bacterial biofilms may be responsible for disrupting this event in the inflammatory phase, which causes chronicity of the wound and keeps healing in an exacerbated inflammatory state[4].

The management of chronic wounds with suspected biofilm is complex because, over time, the microbiology of the biofilm becomes diverse and results in the formation of a structure that is highly resistant to antimicrobial action[3]. The impact on delayed healing has attracted increasing attention from researchers and health care professionals, including nurses. Its importance has led to the establishment of biofilm-based wound care, where chronic wounds are treated using multifaceted strategies in an attempt to remove biofilms from the wound bed to facilitate recovery of epithelial integrity[5].

Microbial biofilms are recognized throughout the scientific community as a cause of wound chronicity[6] and delay in the process of tissue repair[4]. However, there has been frequent discussion about how to evaluate and clinically identify biofilm. There are questions about its management using wound cleansing strategies; including the use of various types of topical treatments for its disruption, eradication, and inhibition of its re-composition in wounds[3]. In this context, we propose the hypothesis that biofilm often forms on the skin wound and interferes with its healing.

In view of the controversies in clinical practice, knowledge of the synthesis of research already published in the literature on biofilm formation in wounds can provide support for nurses and nursing staff in planning and implementing measures that can help identify the presence of biofilm in the wound and adopt strategies for its management. Thus, this study aimed to identify in the literature the biofilm formation and its behavior when faced with interventions in cutaneous wounds.

Methods

This is an integrative literature review. The conduct of the study was guided by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) checklist, including the organization of information. Research steps: development of the research question; literature search for primary studies; data extraction; evaluation of primary studies; analysis and synthesis of results; and presentation of the review[7]. As a starting point, the questions, "How does biofilm formation occur? What is biofilm behavior in the face of skin wound interventions?" In elaborating these, the PVO technique was used[8], where "P" refers to the population, context and/or problem situation of the research (wound biofilm); "V", to the variables of the study (biofilm formation and interventions); and "O", to the desired/achieved outcomes (biofilm behavior and management outcomes).

The search for primary studies was conducted in the Cumulative Index to Nursing and Allied Health Literature (CINAHL), Latin American and Caribbean Literature on Health Sciences (LILACS), EMBASE, Scopus, The Cochrane Library Collaboration (COCHRANE), Medical Literature Analysis and Retrieval System online (MEDLINE) databases through the National Li-

brary of Medicine National Institutes of Health (PubMed) and Science Direct, which is a website operated by the Anglo-Dutch publisher Elsevier.

To perform the search, the researchers selected the following controlled descriptors from Medical Subject Headings (MeSH) and Descriptors in Health Sciences (DeCS) terminology: biofilms, wounds and injuries, leg ulcer, diabetic foot, varicose ulcer, surgical wound, *in vitro* techniques, animal models, clinical protocols. The non-controlled descriptors (keywords) were considered by the researchers to broaden the identification of published studies and were established according to previous readings on the topic of interest: bacterial biofilm, pressure injury, excisional wound. To ensure a broad search, the controlled and non-controlled descriptors were used in different ways, alone and combined with each other, with the Boolean operators AND and OR.

The electronic search in the databases included articles with a focus of investigation on wound formation, or on its behavior in the face of interventions; published until the year 2021, in Portuguese, English and Spanish (Figure 1).

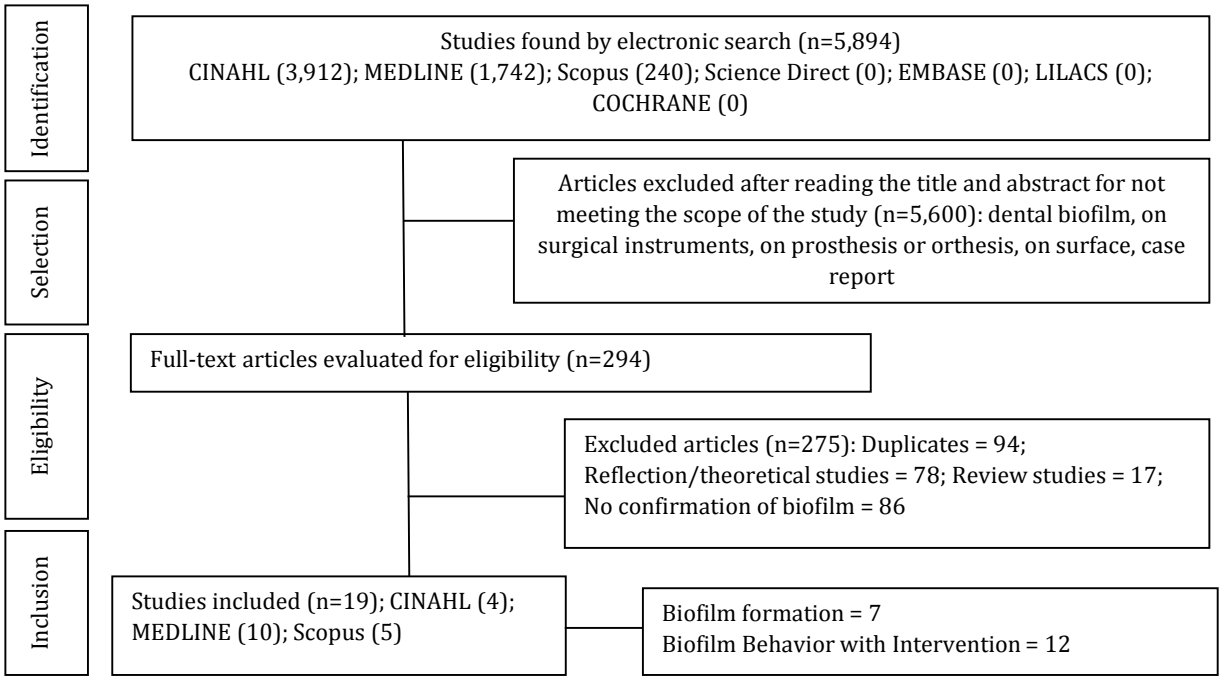

Figure 1 – Flowchart of article selection for the review. Belo Horizonte, MG, Brazil, 2021

The exclusion criteria consisted of studies on dental biofilm, on surgical instruments, on prosthesis or orthesis, on surface. Also excluded were articles identified as reflection/theoretical, editorial or letter-response, commentaries, theses and dissertations. No filters were applied to the publication period of the articles to ensure adequate numbers of primary studies. Articles present in more than one database were excluded from the sample.

The search for the primary studies in the selected databases occurred in the month of July 2021 and was conducted by one of the authors of the present study in conjunction with a librarian. The selection of the articles was carried out by two authors of the review independently. A meeting was scheduled between them to discuss the differences. The same researchers performed the data extraction, also independently, with the aid of an instrument validated by scholars and used by other researchers[9]. The instrument was chosen for its simplicity and for meeting the

peculiarities of the guiding questions of the study. It includes the following information: identification of the publication (article title, language, authors, date of publication), journal of publication, methodological characteristics of the study (type of publication, type of research [*in vitro*; *in vivo* in animal models, *in vivo* in humans], sample/participant, biofilm formation, intervention, results [biofilm behavior]).

To analyze the material, it was decided to group and categorize the biofilm information according to the type of research: *in vitro*; *in vivo* in animal models, *in vivo* in humans. This diversity, together with the research proposal, made it impossible to classify the studies included in the sample in levels of evidence. The analysis of the results was carried out descriptively. Subsequently, comparisons emphasizing the differences and similarities of the studies were performed.

Results

The 19 articles in the sample were published in the period from September 2010 to September 2021, seven of which addressed biofilm formation and 12 the behavior of the biofilm in the face of interventions. Three types of biofilm studies were identified: two clinical, six *in vitro* (Figure 2), and 11 *in vivo* in the animal model (Figure 3). They included three themes: biofilm model creation (n=4); biofilm assessment (n=3); and biofilm behavior in the face of interventions to manage biofilm (n=12). The latter group included negative pressure therapy (n=3); Ag/Fe3O4 nanocomposites (n=1); propolis (n=1); enzymes (n=1); antimicrobial product (n=1); ultrasonic debridement (n=1); coating (n=2); nitric oxide (n=1); tryptophan amino acid (n=1).

Approach/Authorship	Biofilm formation and behavior
Clinical Mori, et al.[5]	In Study 1 the median proportion of biofilm removal was 38.9% for pressure sores treated with standard care and 65.2% for those treated with ultrasonic debridement (p=0.009). In Study 2 the proportion of wound healing was significantly higher in wounds treated with the "biofilm based wound care system" than in those treated with standard care.
Clinical Han, et al.[10]	Tissue samples were obtained from 15 patients with chronic wounds. Standard bacteriological cultures demonstrated an average of three common bacterial species per wound. Pyrosequencing revealed an average of 17 in each wound and the presence of highly organized thick confluent biofilms.
In vitro Guedes, et al.[11]	Biofilms grown ex situ have more bacterial cells and polymeric matrix than *in vitro*, reaching maturity at 72 hours of growth. The production of virulence factors differs between ex situ and *in vitro* biofilms.
In vitro Pirlar, et al.[12]	Minimum concentrations of trypsin, betaglucosidase and DNase I enzymes to degrade biofilms were 1µg/ml, 8 U/ml and 150 U/ml, respectively. The combination of 0.15µg/ml trypsin and 50 U/ml DNase I resulted in the dissolution of all *Staphylococcus aureus* and *Pseudomonas aeruginosa* biofilms.
In vitro Kwiecińska-Piróg, et al[13]	Ethanolic extract of propolis was effective on P. mirabilis biofilm at a concentration of 25-100 mg/mL to prevent its formation and 25-50 mg/mL when mature.
In vitro Tahir, et al.[14]	Negative pressure therapy was applied to the mature biofilm model of *Staphylococcus aureus* and *Pseudomonas aeruginosa* with alteration of its architecture, reducing its thickness and mass. The therapy with instillation of polyhexamethylene biguanide-based solution was highly effective in reducing biofilms of *S. aureus* and *P. aeruginosa*.
In vitro Ghaseminezhad, et al.[15]	The combination of silver nanoparticles (Ag+) with iron oxide (Fe3O4), in the production of Ag/Fe3O4 nanocomposites, was able to penetrate and eradicate *S. aureus* biofilms upon application of a magnetic field.
In vitro Ngo, et al.[16]	Negative pressure therapy applied to the *Pseudomonas aeruginosa* biofilm model showed little (statistically significant) reduction of bacteria within two weeks. The action was much more considerable and observed within 24 hours when the therapy was combined with silver impregnated foam. There was compression of the biofilm architecture with reduction of the thickness and the diffusion distance.

Figure 2 – Distribution of selected *in vitro* and clinical studies with synthesis of biofilm formation and behavior. Belo Horizonte, MG, Brazil, 2021

Approach/ Authorship	Biofilm formation and behavior
Animal model Kim, et al.[17]	High levels of oxidative stress in the wound significantly altered the microbiome, which decreased bacterial diversity and promoted colonization by bacteria from the skin microbiota (*Pseudomonas aeruginosa, Enterobacter cloacae, Corynebacterium frankenforstense* and *Acinetobacter sp.*) and biofilm formation. There tended to be only one bacterium with aggressive biofilm development.
Animal model Stoffel, et al.[6]	Of the commercial products containing topical antimicrobial agents, iodine and benzalkonium chloride were the most effective *in vitro* and underwent *in vivo* evaluation in an immunocompromised murine model with a biofilm wound, resulting in significant biofilm reduction by benzalkonium chloride.
Animal model Davis, et al.[18]	Silver gelling fiber coating on a *Pseudomonas aeruginosa* biofilm model in a pig wound resulted in biofilm shedding.
Animal model Brandenburg, et al.[19]	A *Pseudomonas aeruginosa* biofilm model was created in a burn done on a mouse. The infection caused local and systemic immune responses. *P. aeruginosa* was entangled in an extracellular matrix on the surface of the burn, as well as penetrating 500-600 μm deep into the eschar.
Animal model Hasan, et al.[20]	Nanoparticles of PLGA - poly(lactic-co-glycolic acid) as a polyethyleneimine/diazeniodiolate (PEI/NONOate) nanoparticle-forming poly(lactic-co-glycolic acid) nanoparticles, with the ability to bind to the biofilm matrix, were developed to facilitate the delivery of nitric oxide to *Staphylococcus aureus* biofilm from wounds in mice. The nanoparticles bind tightly to the matrix, resulting in reduced bacterial load and complete dispersion of the biofilm.
Animal model Guoqi, et al.[21]	In the rabbit ear wound biofilm model of *Pseudomonas aeruginosa*, the group treated with negative pressure therapy was compared to the group treated with gauze. Negative pressure therapy resulted in a significant reduction in the expression of all virulence factors tested, including exotoxin A, rhamnolipid, and elastase, and a significant reduction of biofilm components.
Animal model Karna, et al.[22]	The rabbit ear excisional wound model was used to evaluate transcriptomic changes in wounds as they combat *P. aeruginosa* infection. In wounds with infection (biofilm), several types of noncoding RNA (ncRNA) were suppressed after wounding, with increases after five and nine days, suggesting a sequential and coordinated change in transcript levels.
Animal model Brandenburg, et al.[23]	Proposition of a model for biofilm formation of *Pseudomonas aeruginosa* on a commercially available biocover (Biobrana®) and evaluation of the inhibition of *Pseudomonas aeruginosa* biofilm formation by the amino acid tryptophan (D-/L-tryptophan) on (murine) rat wounds. D-/L-tryptophan inhibited aeruginous biofilm formation on the dressing in a dose-dependent manner and was not directly cytotoxic to human keratinocytes. D-/L-tryptophan-treated wound closure increased compared with untreated wounds.
Animal model Seth, et al.[24]	Evaluation of the impact of antimicrobial dressing [AQUACEL Ag+ Hydrofiber Dressing] on rabbit ear wounds infected with *Pseudomonas aeruginosa* biofilm. Compared to the wounds with inactive vehicle (control), there was statistical significance in the improvement of healing and reduction in bacterial count.
Animal model Gurjala, et al.[25]	An animal model of skin wounds on rabbit ears was developed with *Staphylococcus aureus*. Mature biofilm formed within 24 hours. Inflammatory markers confirmed that the biofilm phenotype creates a characteristic, sustained, low-grade inflammatory response and that, over time, biofilm impairs epithelial migration and granulation tissue growth.
Animal model Zhao, et al.[26]	A reproducible chronic wound model was created in diabetic mice by applying bacterial biofilm. No wound with biofilm was closed within 28 days after wounding. There was extensive inflammatory cell infiltration, tissue necrosis, and epidermal hyperplasia adjacent to wounds. Most bacteria were in the crust above the wound bed, not in the wound tissue.

Figure 3 – Distribution of selected *in vivo* studies in the animal model with the synthesis of biofilm formation and behavior. Belo Horizonte, MG, Brazil, 2021

The descriptive analysis allowed us to identify that the time for biofilm formation and its structure vary according to the bacteria involved and the type of *in vitro* and animal model. The clinical studies did not address the time required for biofilm formation.

Some products and solutions were able to reduce or remove the biofilm. These findings were obtained in the *in vitro* and animal model studies. The products that showed these activities were Ag/Fe3O4 nanocomposites, propolis, tryptophan and enzymes, silver coatings, negative pressure therapy with silver or poly hexamethylene biguanide instillation, and ultrasonic debridement.

Discussion

In vitro, *in vivo* (animal) and clinical studies have addressed biofilm in various wounds and bring a growing body of evidence suggesting that bacterial biofilms represent an important source of research for understanding the pathogenesis of chronic wounds[11,25]. This fact was confirmed by most of the studies in the sample of this research, which used the *in vitro* or *in vivo* animal model, and few clinical researches carried out in humans.

The studies used the *in vitro* model to evaluate biofilm formation[11], effect of interventions on the management of biofilm formed by *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *P. mirabilis*. The evaluated interventions involved products and technologies such as negative pressure therapy[14,16], propolis[13], enzymes[12], in addition to the use of nanoparticles to deliver silver[15].

Open or dynamic system biofilm models have similar principle to continuous cultures, usually allow the control of environmental parameters such as shear forces, and therefore have been widely used to study the physical and chemical resistance of biofilms. Microcosm models take into account the complexity and heterogeneity of natural environments[2]. Therefore, they are more sophisticated in mimicking in situ conditions, since they include several bacterial species[14] and use material from the studied environment to simulate an *in vivo* situation[11].

The effect of negative pressure therapy with and without solution instillation was evaluated in the *in vitro* biofilm model[14,16] and *in vivo* in an animal model[21]. The evaluations performed showed a small, but statistically significant reduction of biofilm bacteria within two weeks of treatment. When negative pressure therapy was combined with silver impregnated foam, the reduction was more significant and was observed within 24 hours[16]. The use of negative pressure therapy with solution instillation potentiated the action on the biofilm. The highlight were the antimicrobial solutions, especially those based on polyhexamethylene biguanide[14].

The data obtained may not translate into the same efficacy or results when used clinically *in vivo*. Despite this possibility, the studies provide evidence for the use of negative pressure therapy in the clinic, confirming that the technology is effective in reducing virulence factors and biofilm components. Thus, the results of these studies may explain the increased wound healing in clinical practice when using this therapy[27].

Silver dressings are widespread in clinical practice for the control of critical colonization or wound infection. Studies on silver nanoparticles have drawn attention as an alternative to antibiotics for the treatment of wound infections, but their use is challenged by limited tissue penetration and high cytotoxicity. However, to circumvent these problems, researchers combined silver nanoparticles with iron oxide and produced Ag/Fe3O4 nanocomposites that penetrate and eradicate biofilms upon application over a magnetic field[15]. Therefore, it is highlighted that silver nanoparticles are considered a potential avenue in the development of new therapeutic strategies for the treatment of chronic wound infections.

Another *in vitro* biofilm model, with Proteus mirabilis, was used to evaluate the activity of the ethanolic extract of propolis. It was identified that concentrations of 25-100mg/mL of the ethanolic extract of

propolis inhibited the formation of Proteus mirabilis biofilm; and concentrations of 25-50mg/mL acted on the mature biofilm[13]. However, this study presents a strong limitation, which is the absence of a description of the methods used for the evaluation of the outcome under the action of the product.

Another strategy employed in research is the attempt to defeat the biofilm by destroying the self-produced extracellular polymeric substance that surrounds the component bacteria of the biofilm. This structure results in high tolerance to antibiotics, predisposition to chronicity of infection, and complication of wound healing[11-12].

It is important to highlight that most antibiotics cannot remove biofilms in chronic infections, so new therapeutic modalities are needed to promote the breakdown of extracellular polymeric substance. Therefore, the degradation of this structure by the action of enzymes can be used to result in improved healing of chronic wounds[11-12].

One study made use of three enzymes: trypsin, betaglucosidase, and deoxyribonuclease I (DNase), which target the major components of the biofilm. The effectiveness of these enzymes was proven in degrading *Staphylococcus aureus* and *Pseudomonas aeruginosa* biofilms in the wound-like medium, along with reducing the minimum concentration of the antibiotics meropenem and amikacin for biofilm eradication[12]. These findings signal the need to deepen the knowledge in search of building similar and safe proposals for clinical practice.

Several biofilm models have been used to evaluate products containing topical antimicrobial agents, commonly used in wound care by practitioners in clinical practice[6,12,15]. The antimicrobials had distinct performances according to the biofilm model[6]. Changing the microbial growth conditions or combination of organisms resulted in significant differences in performance for some treatments, without confirming the effectiveness of the solutions commonly used in clinical practice.

There are several topical products with antimicrobial action available for wound treatment, whose efficacy has been routinely demonstrated with planktonic microorganisms. However, to date, there is no reference value to support the antimicrobial efficacy of wound care products in biofilm models. In addition, the data for antimicrobial efficacy show varying test methods[6]. Despite this fact, some health care facilities have adopted such solutions with the intention of managing biofilm in chronic wounds.

The studies found on *in vivo* biofilm, those that include animals, range from the proposed validation of an animal model to the evaluation of product efficacy in biofilm elimination. They involved mice[17,20,26], rats[6,23], rabbits[2122,24-25] and pigs[18], under the action of various pathogens, especially *Pseudomonas aeruginosa* and *Staphylococcus aureus*, in inducing biofilm formation.

In vivo model validation is essential for understanding the mechanism of biofilm formation and for evaluating products used in its prevention and management. *In vivo* biofilm models of traumatic wounds in animals include skin abrasions, burns, and lacerations, surgical and excisional wounds, developed in rabbits, rats and mice. The most commonly studied microorganisms associated with wound infections are *S. aureus* and *P. aeruginosa*[2].

Chronic wounds, especially in diabetic patients, are characterized by high levels of oxidative stress resulting from the bacteria that form the biofilm, which prevents healing in a short time. This fact was the basis of a study in a diabetic rat model, whose results confirmed this fact[17]. The reproducible chronic wound model in diabetic mice had *Pseudomonas aeruginosa*-induced biofilm formation, and histological analysis showed extensive inflammatory cell infiltration, necrotic tissue and adjacent epidermal hyperplasia[26].

Another study presented an *in vivo* biofilm infection model developed on scald burns in rats. In this study, *Pseudomonas aeruginosa* were spread on the wound surface, and the presence of biofilm was observed on approximately 10^9 colony forming units of burn tissue in seven days. *P. aeruginosa* infection

caused local and systemic immune responses demonstrated by changes in systemic neutrophil counts, histology, and myeloperoxidase activity. Scanning electron microscopy of the rat burn sample confirmed the presence of *P. aeruginosa* in an extracellular matrix at 500-600 µm depth relative to the lesion surface [19]. This finding characterizes that the biofilm formation does not occur on the surface, and this leads to reflection on the effectiveness of interventions involving the use of solutions for removing it.

The rabbit biofilm model used *Staphylococcus aureus*, and the results revealed that within 24 hours of inoculation, this bacterium quickly took the form of mature biofilm on wounds. Biofilm creates a characteristic inflammation that impairs granulation tissue growth and epithelial migration over time[25].

Subsequently, the validated rabbit biofilm model was used to evaluate the impact of silver hydrofiber dressing impregnated with benzethonium chloride and ethylenediaminetetraacetic acid (EDTA) on wounds with *P. aeruginosa* and *Staphylococcus aureus*. The study showed a consistent decrease in bacterial counts as well as improved wound healing when compared to controls[24]. This study represents the first quantifiable and consistent *in vivo* evidence of the impact of a topical antimicrobial dressing against established wound biofilm. However, while this study provides consistent evidence, it still requires clinical trials to evaluate effectiveness in wound treatment in humans.

The hydrofiber dressing with silver was also compared to the gelling fiber dressing with silver in a porcine wound biofilm model. *Pseudomonas aeruginosa* was inoculated into these wounds, which were then covered with polyurethane film. This study showed that the silver gelling fiber dressing treatment significantly reduced *P. aeruginosa* biofilm when compared to all other treatment groups[18].

The efficacy of five silver gelling fiber coatings was evaluated on biofilms, single species and multi-species, in *in vitro* models. Biofilms of Staphylococcus aureus, Pseudomonas aeruginosa and *Candida albicans* were used. In the single-species models, all five coatings were effective in eradicating the biofilm bacteria with *Staphylococcus aureus* and *Pseudomonas aeruginosa*. However, only one dressing (hydrofiber technology with combined anti-biofilm/antimicrobial technology) was able to eradicate both multispecies biofilms such that no viable organism was recovered and single-species biofilm of Candida albicans[28].

Considering that these dressings are used by professionals, including Brazilians, to treat patients with wounds, this evidence is important to support the indications in practice. Thus, it is noteworthy that these studies on hydrofiber dressings have generated knowledge to support clinical practice, especially in the management of wounds critically colonized by gram-negative biofilm-forming bacteria.

A biological dressing commercially available in Brazil, classified as a biosynthetic skin substitute, supported the biofilm formation of *P. aeruginosa* to evaluate the action of D-/L-tryptophan. It was found that this did not impair wound healing, and there was even improvement[23]. These results demonstrate tryptophan's ability to inhibit bacterial biofilm formation. This indicates the benefit of this component in the development of new products or for its inclusion in existing coatings.

The healing process of chronic wounds, especially venous ulcers, pressure sores and diabetic foot ulcers, usually evolves to a state of chronicity. This situation can be explained by the inefficient eradication of infectious and opportunistic pathogens[27]. A comprehensive description of the microbial characteristics of chronic wounds requires refined investigative techniques, because such wounds contain complex polymicrobial communities of sessile organisms. The results of examinations performed when the chronic wound shows signs of infection or critical colonization are underestimated due to the limitations of standard culture technology[10].

One proposal for biofilm identification is the

use of wound blotting, developed to visualize the biofilm in a fast and non-invasive way. This technique can direct ultrasonic debridement, which is a method already available for biofilm removal. A study was performed to investigate the effectiveness of the biofilm-based wound care system, confirming that the proportion of 90-day wound healing was significantly higher in wounds treated with the system compared to those receiving standard care[5]. This evidence indicates that the proposed system may be a promising therapeutic strategy to visualize biofilms; however it requires further knowledge to be incorporated in other realities.

Studies with *in vitro* models have limitations, most notably the failure to reproduce the host environment. This has led to the rapid development of multiple *in vivo* models, which also have their limitations due to the use of animals, with ethical issues being the main barrier. This partially explains why non-mammalian *in vitro* and *in vivo* surrogate models are still widely used and continue to reveal important information on biofilm physiology and promising treatments for biofilm management.

Data on the antimicrobial activity of products in biofilm models are diverse by many test methods in a variety of studies. There are several topical wound care antimicrobial products available for use, however their efficacy has been routinely demonstrated with planktonic microorganisms.

The results obtained from the review identified the gaps that still exist in the formation of wound biofilms in humans, as well as the financial and ethical limitations to the development of clinical studies. There is a need to develop studies to evaluate molecular diagnostic tools that can be used in clinical practice to identify the compositions of bacterial communities present in pathogenic biofilms of chronic wounds and infections. It is important to consider that microbial biofilms have become increasingly recognized as a cause of wound chronicity. However, there is no target reference value for antimicrobial efficacy of wound care products in biofilm models.

Study limitations

The diversity and methodological frailties of the primary studies led to limitations in the study, making it difficult to automatically transfer knowledge to clinical practice. The review had a heterogeneous sample, composed of *in vivo* and *in vitro* studies. Some studies did not report the number of samples evaluated or the step-by-step preparation of the material. In addition, certain studies did not rely on electron microscopy to identify the biofilm.

Contributions to practice

The limitations presented did not prevent the construction of knowledge for clinical practice. The knowledge gained from the analysis of studies *in vitro* and in animal models can contribute to a better understanding of the mechanisms in the biofilm formation and its identification. Certain technological solutions are indicative for the management of biofilm in the wound, which can instrumentalize the nurse in decision making.

Conclusion

Biofilm formation and behavior were identified through literature review. The detrimental effects of biofilm on wound healing have been confirmed. Several interventions were able to reduce and eliminate biofilm in *in vitro* and *in vivo* (animal) models, so rigorous models are increasingly needed to investigate the efficacy of antimicrobial products, including dressings.

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Authors' contributions

Conception and design and article writing: Amorim GL.

Analysis and interpretation of the data and relevant critical review of the intellectual content: Spira JAO, Coelho ACSM.

Conception and design, data analysis and interpretation, article writing, and final approval of the version to be published: Borges EL.

Agreement to be responsible for all aspects of the manuscript related to the accuracy or completeness of any part of the work to be properly investigated and resolved: Borges EL.

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