Journal of Infection 80 (2020) 261–270

Contents lists available at ScienceDirect

Journal of Infection

journal homepage: www.elsevier.com/locate/jinf

The efficacy of topical agents used in wounds for managing chronic biofilm infections: A systematic review

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ARTICLE INFO

Article history:
Accepted 24 December 2019
Available online 31 December 2019

Keywords:
Chronic wound
Infection
Biofilm
Topical agent

SUMMARY

Objectives: Clinicians have increasingly adopted the widespread use of topical agents to manage chronic wound infections, despite limited data on their effectiveness *in vivo*. This study sought to evaluate the evidence for commonly employed topical agents used in wounds for the purpose of treating chronic infections caused by biofilm.

Method: We included *in vitro*, animal and human *in vivo* studies where topical agents were tested for their efficacy against biofilms, for use in wound care. For human studies, we only included those which utilised appropriate identification techniques for visualising and confirming the presence of biofilms.

Result: A total of 640 articles were identified, with 43 included after meeting eligibility. *In vitro* testing accounted for 90% ($n = 39$) of all included studies, five studies using animal models and three human *in vivo* studies. Sixteen different laboratory models were utilised, with the most frequent being the minimum biofilm eradication concentration (MBEC™) / well plate assay (38%, $n = 15$ of 39). A total of 44 commercially available topical agents were grouped into twelve categories with the most commonly tested agents being silver, iodine and polyhexamethylene biguanide (PHMB). *In vitro* results on efficacy demonstrated iodine as having the highest mean log10 reductions of all agents (4.81, ±3.14).

Conclusion: There is large disparity in the translation of laboratory studies to researchers undertaking human trials relating to the effectiveness of commercially available topical agents. There is insufficient human *in vivo* evidence to definitively recommend any commercially available topical agent over another for the treatment of chronic wound biofilms. The heterogeneity identified between study designs (*in vitro* to *in vivo*) further limits the generalisability of results.

Introduction

Biofilms are frequently defined based on *in vitro* observations. Classic definitions often describe biofilms as; aggregates of microorganisms embedded in a matrix of extracellular polymeric substances (EPS).[1–6] *In vivo*, biofilms can be attached to host or foreign body surfaces or exist in fluids adjacent to those surfaces, or lodged in tissue.[7,8] Microbial cells in a biofilm exhibit altered growth and behaviours which make them highly tolerant towards antibiotics and host defences.[9–11] This explains why biofilms have been acknowledged as a growing concern in healthcare. In chronic wounds they have been implicated as a cause of delayed healing,[12] and as drivers of chronic and persistent infections.[13,14]

Topical antimicrobials and antiseptics (and non-antimicrobial agents) for treating wound infections have been used in healthcare for generations, going back to ancient times.[15] A topical application possess several advantages to systemic therapy including (1) delivering a high dose of the agent at the local site in varying formula-

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https://doi.org/10.1016/j.jinf.2019.12.017
0163-4453/

ries; (2) reduced co-lateral toxicity often associated with systemic therapy, and; (3) an easier administration of treatment by a range of health professionals and the patient themselves. Non-antibiotic topical agents may also prove helpful in addressing the increasing global problem of multidrug-resistant organisms by reducing the use of antibiotics.[16]

The most commonly used topical agents in wounds today have traditionally been employed (and their effectiveness tested *in vitro*) for use against rapidly growing planktonic cells (free floating single cells) that are responsible for causing acute infections. However, as the evidence base has increased for the role of biofilms as a cause of delayed wound healing[12,13,17,18] and as cause of chronic and persistent infections,[14,19,20] these same topical agents have been adopted for use towards managing chronic wounds complicated by biofilm. The inherent problem with this approach has been the lack of evidence available to clinicians from medical device companies with reference to the efficacy of their topical agent in an appropriately designed laboratory model or *in vivo* study.[21] Malone and colleagues[21] published a recent commentary on the lack of laboratory standardisation across testing the efficacy of agents against biofilm. The commentary also highlighted the challenges facing clinicians when relying solely on *in vitro* data, in that many *in vitro* model designs are not necessarily reflective, nor representative of a topical agents intended clinical purpose.

The objective of this systematic review was to collate a comprehensive review of available studies (*in vitro*, animal and human *in vivo*) testing the efficacy of topical agents against biofilms, in the context of that agent being commonly utilised in wounds.

Methods

The systematic review was registered on the PROSPERO database (CRD42018116905) which adhered to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis Protocol (PRISMA).[22]

Eligibility

Test agent

For the purpose of our review we defined a test agent as being an; antiseptic, antimicrobial or surfactant suitable for application on human skin and soft tissue through defined delivery vehicles; gels, ointments, creams, pastes, water or alcohol-based solutions, powder and dressing materials (bio-absorbable/biodegradable). To ensure relevance for clinicians, the decision was made to only include commercially available agents used in wound care. The topical agent must have also held regulatory approval for use as a medical device (wound dressing) from an appropriate regulatory body (*i.e.* Food and Drug Administration [FDA] or Conformite Europeene [CE] mark). This excludes studies testing extractions from dressings, rather than the available product. We did not include novel agents, peptides, compounds or any other agents used outside of their intended application (*i.e.*, off-label use).

Inclusion and exclusion criteria

We included *in vitro*, animal (*ex vivo* and *in vivo*) and human *in vivo* studies. For studies to be considered eligible, the topical agent being tested must have been a product that was commercially available for use in human wounds. Study designs which sought to assess the effectiveness of topical agent/s in eradication or reduction of established biofilms were included. We did not include study designs where a topical agent was tested against the ability to prevent biofilm formation through its activity against planktonic phase bacteria (biofilm forming strains). Studies focused on topical antibiotics or disinfectants were excluded as their routine use in wounds is not recommended.[23,24] For human *in vivo* studies we included randomised and non-randomised controlled trials, case-control studies, cohort studies, before-and-after studies and prospective non-controlled studies. We did not include retrospective studies, case studies or conference abstracts. For human *in vivo* studies all participants must have been over 18 years and treated with a topical agent for a lower limb wound. This included diabetic foot ulcers (DFU), venous leg ulcers (VLU), pressure ulcers (PU) and non-healing surgical wounds (NHSW). We did not include burns or other connective tissue, inflammatory or blistering diseases as the pathology of these wounds is vastly different, and the management strategies have their own unique considerations. Additionally, only studies that used appropriate visualisation techniques to confirm the presence of biofilm in human tissue were included (scanning electron microscopy [SEM], transmission electron microscopy [TEM], conventional and peptide nucleic acid fluorescent *in situ* hybridisation [PNA-FISH] with confocal laser scanning microscopy [CLSM], light microscopy and transmission).

Search strategy

A systematic search of the literature was undertaken between the 26th of November 2018 and 10th of December 2018 using a pre-defined search mesh (S1). Articles published after the year 2007 were eligible for review, and articles of any language were included. The Cochrane Library, Pubmed/Medline, EMBASE and clinical trial registries were systematically searched. All titles and abstracts were imported into Zotero reference management software (Zotero 5.0, Fairfax, Virginia, USA). Titles and abstract were screened independently by two authors (SS & MM).

Data extraction and quality assessment

Data extraction and the quality of included studies were independently assessed by the panel of authors. A data extraction and grading tool to assess the quality of *in vitro* studies and collate the evidence was created by the authors as there were none publicly available (S2). The tool is an adaptation of the 'minimum information about a biofilm experiment' (MIABiE) initiative.[25] No publicly available and accepted bias assessment tool for *in vitro* studies exists. Animal studies were graded using the ARRIVE guidelines (Animal Research: Reporting of *In Vivo* Experiments), and allocated a score corresponding to the recommendations.[26] Human studies were graded using the Scottish Intercollegiate Guidelines Network (SIGN).[27] The extracted data for all models can be viewed in S3.

Outcomes of interest

The primary outcome measure was to collate the overall evidence available on the quality and level of evidence for the effectiveness of topical agents against biofilms in the context of human wounds. Specifically, we sought to establish which types of topical agents had been tested, and in which study designs, in order to understand where the majority of evidence exists. This may highlight if there are suitable levels of evidence for any agent/s across a range of testing methods (*in vitro* to human) and identify any gaps for future research needs.

For the purpose of this study, we defined effectiveness of a topical agent in relation to its effects on biofilm; killing microbes in biofilm, removing/dispersing biofilm and reducing EPS. Secondary outcomes of interest included; reporting study log reductions (colony forming units measured in millilitre or gram [cfu/ml/or g]) or other measures of bacterial activity (*i.e.* quantitative polymerase chain reaction (qPCR), biomass or optical density) to identify if any topical agents performed better than others when viewed collectively, and review and assessment of laboratory and animal model designs and within study variables to provide an overall quality score. For human *in vivo* studies, the evidence level was assessed, and data extracted to identify if topical agents are

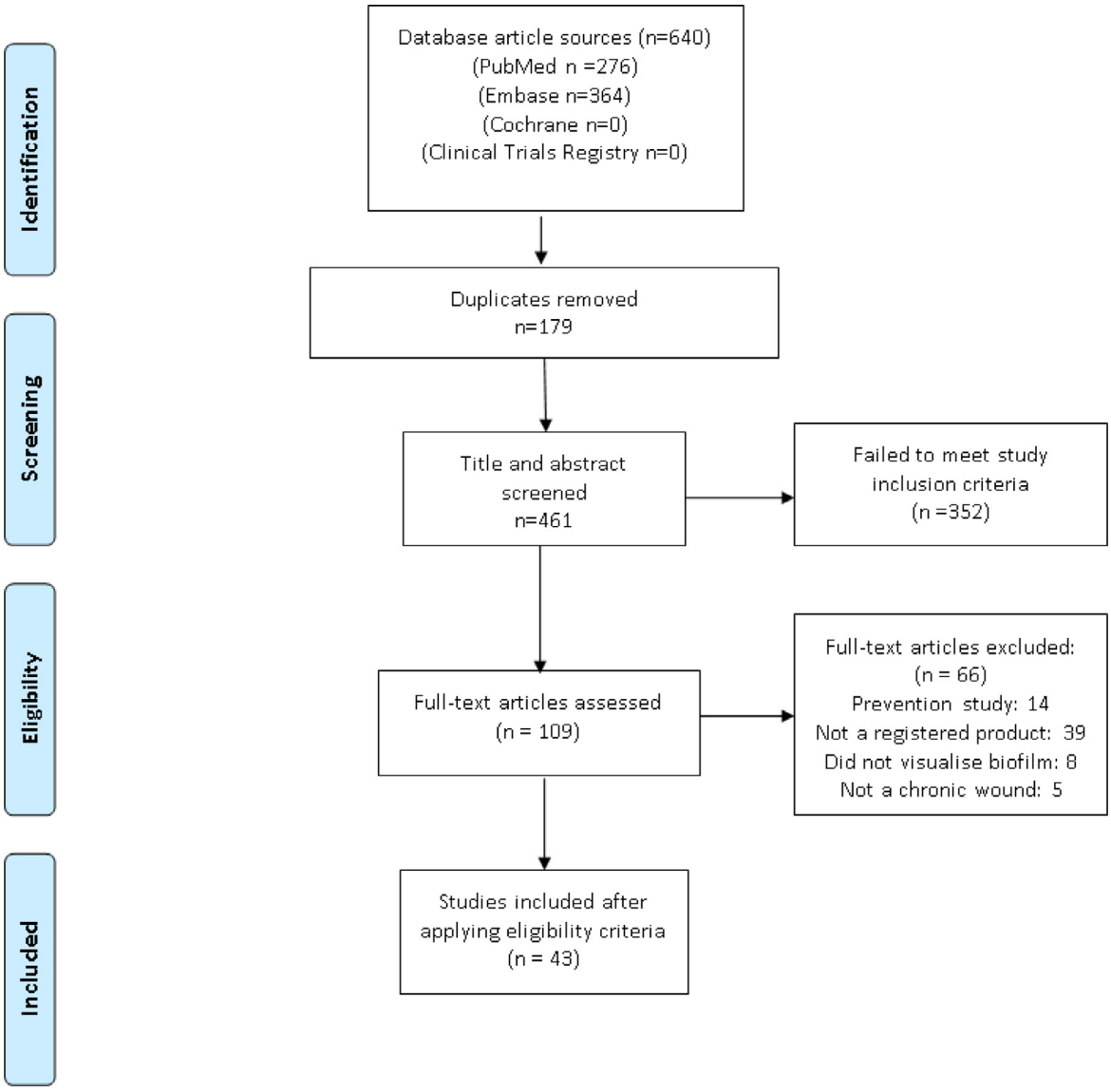

Fig. 1. PRISMA flow diagram mapping out the number of records identified, included and excluded, and the reasons for exclusions.

effective against wound biofilms, and if their use impacts wound outcomes.

Statistics

A narrative synthesis approach was used to summarise data extracted from full-text articles. Meta-analysis was not possible due to the heterogeneity of the data. Bacterial reduction data measured by cfu/ml/or gram was standardised by log transforming the data, if not already presented in this manner by the author. Data were analysed through Statistical Package for Social Sciences Version 24 (SPSS Inc., Chicago, Illinois, USA). For all comparisons and modelling, the level of significance was set at $P< 0.05$. Data are given as mean, range and standard deviation (±).

Results

A total of 640 articles were identified. Fig. 1 depicts the PRISMA flowchart of included studies and reasons for study exclusion. After removal of duplicates, 461 articles were screened for inclusion. After screening each articles title and abstract, a further 352 were excluded for not meeting eligibility criteria. The full text of 109 articles were assessed, and a further 66 articles were excluded. In total, 43 articles (47 studies) were included for review; 39 *in vitro* studies, five animal studies, and three human *in vivo* studies (S3). Four papers used more than one approach (*in vitro*, animal and human *in vivo*). The different models that these studies utilised are shown in Table 1.

Formulary of topical agents

A total of 44 commercially available topical agents were grouped into twelve categories shown in Table 2. The most commonly tested agents were; silver agents ($n = 18$), tested in sixteen *in vitro* models,[28–44] two *in vivo* mouse models and a rabbit ear model.[31,40,44] Iodine agents ($n = 5$), tested in twelve *in vitro* models,[31,33,36,38,39,41,45–50] one *in vivo* mouse model[31] and one human *in vivo* study. [51] Polyhexamethylene biguanide (PHMB) agents ($n = 3$) were tested in nine *in vitro* models,[31,36,39,45,46,48,52–54] an *in vivo* porcine study,[55] and one human *in vivo* study.[56]

Table 1
Represents all Biofilm Models used in included studies.

Model	Number of times used and study reference:	
In Vitro Models		
96 Well plate/MBEC/Well plate/microtiter	15	Klasinc et al. 2018; Halstead et al. 2017; Ortega-Pena et al. 2017; Percival et al. 2017; Zamora et al. 2017; Bowler et al. 2016; Desroche et al. 2016; Bjarnsholt et al. 2015; Halstead et al. 2015; Larko et al. 2015; Cooper et al. 2014; Gawande et al. 2014; Lu et al. 2014; Brackman et al. 2013; Kostenko et al. 2010
CDC reactor	6	Johani et al. 2018; Tahir et al. 2018; Hoekstra et al. 2017; Percival et al. 2017; Touzel et al. 2016; Ngo et al. 2012
Filter	3	Percival et al. 2017; Bjarnsholt et al. 2015; Wu et al. 2015
Continuous Flow Tube reactor	1	Sauer et al. 2009
Flatbed perfusion	2	(a)Thorn et al. 2009; (b) Thorn et al. 2009
Drip flow reactor/Colony DFR	2	Bourdillon et al. 2017; Fitzgerald et al. 2017
Colony Biofilm Model	1	Fitzgerald et al. 2017
Porcine Explant	5	Johani et al. 2018; Yang et al. 2018; Yang et al. 2017; Phillips et al. 2015; Phillips et al. 2013
Glass chamber slide	2	Klasinc et al. 2018; Percival et al. 2008
Filter disc	2	Parsons et al. 2016; Banerjee et al. 2014
Polystyrene plate	1	Day et al. 2017
Microscope slide	1	Vestby and Nesse 2015
Semi-solid	1	Crone et al. 2015
Ampule	1	Said et al. 2014
Constant depth film fermenter	1	Hill et al. 2010
Agar plate (gauze implanted)	4	Kalan et al. 2017; Bowler et al. 2016; Parsons et al. 2016; Seth et al. 2014
Animal Model		
Rabbit Ear	1	Park et al. 2016
Porcine	1	Davis et al. 2017
Mouse	1	Fitzgerald et al. 2017; Gawande et al. 2014
Human		
Venous Leg ulcer	1	Borges et al. 2018
Diabetic Foot Ulcer	2	Johani et al. 2018; Malone et al. 2017

Table 2
Topical agents identified by generic agent and brand.

Agent	Brand (or generic ingredient if un-branded)	Vehicle/s of delivery
Silver	Acticoat™, Silverlon®, Aquacel® Ag, Aquacel® Ag Extra™, Silvercel™, Polymem® silver, GranuFoam Silver™ (VAC dressing), Promogran Prisma™, Urgoclean Ag, Atruman® Ag, Mepilex® Ag, Tegaderm® Ag, Askina® Calgitrol, Actisorb silver™, Biatain® Ag, Silvasorb®, Therabond®, Exsalt®	Polyurethane dressing, woven nylon, hydrofiber, non-woven pad, granufoam, alginate, poly-absorbent fibres, ointment coated tulle, foam, silicone dressing, alginate matrix, gel, ag oxysalt
Honey	Manuka Honey UMF™, Medihoney®, Surgihoney™, L-Mesitran®	Ointment, gel
Iodine	Cadexomer Iodosorb™, Iodoflex™, Inadine™, Isobetadine®, Braunol®	Solution, hydrogel, ointment, knitted viscose fibres
Polyhexamethylene Biguanide (PHMB)	Prontosan®, Curity AMD™	Gel, solution, non-woven sponge
Poloxomer 188	PluroGel®	Gel
Superbabsorbent polymer	Sorbact®, Sorbion®	Acetate fabric dressing pad
Melaleuca oil	Woundaid®	Solution
Hypochlorous / Acetic acid	Acetic Acid, Vashe® Wound Solution, Superoxidised solution, Microdacyn®, Microcyn®	Solution
Pyridine	Octenidine®, Octenisept®, Octenilin®	Solution
Chlorhexidine	Chlorhexadine, Chlorhexadine and Cetrimide	Solution
Ringers Solution	Ringers Solution	Solution
Electroceutical	Procellera	Single layer of embedded matrix of moisture-activated microcell batteries

In vitro *studies*

In vitro testing accounted for 90% ($n = 39$) of all included studies, with thirty-two study designs using only one model, and six studies employing two or more biofilm models.[32,34,48,52,57,58] Sixteen different laboratory models were utilised, with the most frequent being the minimum biofilm eradication concentration (MBEC™) / well plate assay (38%, $n = 15$ of 39). In ten studies (26%, 10 of 39), this assay was used as the sole laboratory model. Modifications to a laboratory model to better reflect a wound environment (*i.e.* media to mimic wound fluid, replacing media daily) was undertaken in over half of studies (56%, 22 of 39).

In total, seventy-eight different biofilm forming bacteria were used in monospecies models (S4), with the most common species being *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Multispecies models were used in only five studies (13%, 5 of 39).[34,38,58–60] Twenty-four-hour biofilms (growth duration) were most commonly used to test topical agents (46%, 18 of 39) followed by, ≥ seventy-two- hour biofilms (38%, 15 of 39) and forty-eight-hour biofilms (33%, 13 of 39). The most frequently used method to confirm biofilm growth pre-testing was CLSM (36%, 14 of 39). In seventeen studies, biofilm growth pre-testing was not visualised by any microscopy method, with biofilm growth assumed (43%, 17 of 39).[29,36,37,39,40,47,50,52,54,58,59,61–66] A control or untreated sample was used in thirty-two studies, with seven studies having no study control.[28,32,34,37,44,52,63] The most frequent control was an untreated sample, (51%, 20 of 39) while a positive and negative control was used in seven studies (18%, 7 of 39).[54,64–69]

Bacterial inoculation was reported as cfu/ml in all but four studies (10%, 4 of 39).[30,40,50,68] The average inoculation amount was $10^{\wedge 6}$ cfu/ml (±1.09, range: $10^{\wedge 5}$ – $10^{\wedge 8}$ cfu/ml). The harvest of bacteria for quantification was not reported in six studies (15%, 6 of 39), [34,52,62,63,67,70] and most studies used different methods of harvesting; vortex, stomaching, sonication or a combination

Table 3
Data obtained from ≥3 studies on topical agents with the highest performing agents* denoted and subsequent values (log reduction cfu/ml). Performance data reported from 24-hour exposure time point and combined average reporting of all exposure timepoints conducted in the study. (a) *In vitro* study values and (b) all animal model values. Standard deviation is calculated if multiple studies for the same agent are included in the review.

(a)

Agent/Dressing Group		Mean log10 cfu/ml reduction	
		24 h	Combined average
Silver	*Acticoat 7	4.1 (±0.9)	3.73 (±2.05)
	Aquacel Ag	1.39 (±1.29)	1.43 (±1.06)
	All silver agents	2.18 (±1.81)	2.82 (±2.13)
Iodine	*Cadexomer Iodoflex	7.33 (±1.56)	7.4 (±1.3)
	Cadexomer Iodosorb	6.68 (±3.1)	6.42 (±2.67)
	All Iodine agents	4.81 (±3.14)	5.05 (±2.98)
PHMB	*Prontosan solution	4.76 (±2.02)	4.76 (±2.16)
	All PHMB	3.33 (±2.28)	3.47 (±2.42)
Poloxomer 188	*Plurogel	3.71 (±2.37)	4.09 (±2.38)

(b)

Agent Group		Mean log10 cfu/ml reduction	
		24 h	Combined mean
Silver	Acticoat 7		0.1
	*Aquacel Ag	1	1
	Silvasorb	0.7	0.7
	All silver agents	0.85 (±0.21)	0.6 (±0.45)
Iodine	Cadexomer Iodosorb	4.5	4.5
PHMB	Prontosan solution		2.37 (±0.99)
Pyridine	Octenelin		1.66 (±1.27)
	Octenidine		1.82 (±1.16)
	All pyridine		1.74 (±1)
Hypocholous Acid	Hypochlorous acid		0.67

of methods. Nine studies used a neutralising agent against the active agent (23%, 9 of 39) (*i.e.* sodium thioglycolate for silver).[28,29,31–33,43,50,57] Eight studies sonicated bacteria, ranging from 30s to 30min, while some studies did not report the duration (20%, 8 of 39).[28,30,39,46,47,53,60,69] Five studies vortexed bacteria,[29,31,33,38,50] and five studies used both methods (13%, 5 of 39).[36,40,48,57,66] Serial dilution and plating was the most frequent method for quantification if reported (59% 23 of 39). Other methods included surface drop count[68] and the Miles-Mosra enumeration method, used in one study each.[59]

The exposure time or contact period of test agents in biofilm models averaged twenty-four hours which occurred as a singular exposure event, ranging from three seconds to 168h. Two studies tested multiple exposure events in the same biofilm model by testing the topical solution as part of instillation and dwell with the use of negative pressure wound therapy.[45,46] Following exposure, the efficacy of topical agents was primarily measured by bacterial viability (cfu) (82%, 32 of 39). Other methods included; optical density (26%, 10 of 39) and biofilm biomass (20%, 8 of 39). Nine studies reported multiple methods of bacterial viability (23%, 9 of 39).

Twenty-four studies (61%, 24 of 39) included methods of "within experiment" repeats (*i.e.* triplicates). No studies specifically reported experimental designs to incorporate independent repeats, defined as repeated experiments on different days starting the experiment anew, or any reproducibility testing, defined as "between laboratory" repeats of the same experimental design.

Mean log10 cfu/ml reductions were extracted (where possible) for *in vitro* studies in order to demonstrate any trends in effectiveness between both individual agents, and the categories of different topical agents (*i.e.* iodine, silver, honey, surfactants) (S5 and Table 3). This was reported for 24 h as the most common exposure period, as well as the combined average total exposure time. To provide a more robust measure of effect, only agents that had been tested in three or more studies were included in the examination of the highest log reduction values. *In vitro* results on efficacy demonstrated iodine agents as having the highest mean log10 reduction of all agents (4.81, ±3.14), with the highest performing individual agent identified as Cadexomer Iodoflex™ (7.33, ±1.56). Silver agents demonstrated mean log10 reduction of 2.18 (±1.81), with the best performing silver Acticoat™ 7 (4.1, ±0.9). PHMB agents demonstrated mean log10 reduction of 3.33 (±2.37), with Pronotosan® solution the best performing agent (4.76 (±2.02). Poloxomer 188 was also identified as an agent type tested in multiple studies (3.71, ±2.37, Plurogel®).

The aforementioned areas of *in vitro* laboratory design were factored in calculating the mean score of studies using the adapted MIABiE tool. The mean score across all studies was nine out of twenty-two (± 3.57, range 2–16, Grade of evidence: Low) (S3).

Animal studies analysis

Five *in vivo* animal studies ($n = 5$ of 43, 11%) were identified testing the following agents against wound biofilms; silver,[40,44] cadexomer iodine, octenidine dihydrochloride, octenilin, ringers solution, hypochlorous acid,[31] PHMB[55] and honey.[71] The ARRIVE guidelines[26] were used to score the methodological quality of each included study, with the recommendation items scored out of twenty.

Park and colleagues[71] used a rabbit ear wound biofilm model to test the efficacy of Manuka honey against a single species (*S. aureus* UAMS-1, clinical strain) three-day mature biofilm. Tissue punch biopsies were obtained pre and post treatment in addition to desiccation scabs formed during the treatment period. Manuka honey was applied to wounds every second day for six days. SEM and cfu/g tissue were undertaken in the study, but the reporting of both outcomes against a baseline value was unclear (ARRIVE score of 9/20).

Davis and colleagues[55] used a porcine model to test PHMB, octenidine dihydrochloride, octenilin, ringers solution and hypochlorous acid. Six partial thickness wounds were inflicted onto the paravertebral and thoracic area of porcine. The wounds were inoculated with a biofilm forming methicillin-resistant *S. aureus* laboratory strain (MRSA ATCC 33,593) for a twenty-four-hour growth period. Confirmation of biofilm growth was not visualised through microscopy in either control or treatment tissue specimens prior to treatment. The frequency of wound irrigation via the test agents was performed twice daily. At day three and six, tissue punch biopsies were obtained identifying that wounds treated with PHMB exhibited the greatest reduction in bacterial counts (initial bacterial count = 7.4 log10 cfu/g, ±0.49, day three = 5.7, log10 cfu/g, ±0·47, day six = 4.3 log10 cfu/g, ±0.67) (ARRIVE score of 14/20).

Fitzgerald and colleagues[31] used a mouse model to test the performance of a silver gel, silver solid dressing and cadexomer iodine medicated sheet. A biofilm forming multi drug resistant laboratory strain (MRSA ATCC 33,592) was allowed to form for twenty-four hours. Wounds were treated once daily for two days. The results identified cadexomer iodine achieved >4 log10 cfu/g of tissue, compared to a silver solid dressing (1 log10 cfu/g tissue) and silver gel (0.7 log10 cfu/g tissue) (ARRIVE score of 10/20).

Gawande and colleagues[40] used a chronic wound mouse model to test the efficacy of a silver solid dressing against biofilm. A surgical excision wound was inflicted on the back of mice and inoculated with an MRSA clinical isolate and allowed to grow for twenty-four hours. No description was provided as to the ability of the clinical isolate to form biofilm, nor was a pre-test microscopy performed to confirm the presence and growth of biofilm. The results identified a 0.1 log10 reduction in cfu/g tissue with a silver solid dressing (pre-treatment cfu/g tissue = 6.9 log10 *versus* post-treatment = 6.8 log10). (ARRIVE score of 5/20).

Seth and colleagues[44] used a rabbit ear model to test the performance of a silver solid dressing against wild-type seventy-two hour *P. aeruginosa* biofilm. Full thickness dermal wounds were inflicted on the ventral surface of twelve rabbits, with two treatments tested per rabbit, consisting of an 'active control' of PHMB dressing, 'inactive' alginate dressing and the silver solid dressing. An *in vitro* model was used to examine biofilm growth on gauze with SEM, however no imaging was undertaken on the rabbit ears. The results illustrated a two log10 cfu/wound after three applications of silver solid dressing every two days, compared to both the active and control dressing. (ARRIVE score of 11/20).

The mean log10 cfu/g reductions from the five studies were extracted where possible and are shown in Table 3b. The animal data showed an overall trend of agents demonstrating lower efficacy against biofilm compared to the *in vitro* results. Iodine demonstrated the highest log10 reductions, (4.5 log10 Cadexomer Iodosorb™) followed by PHMB (2.37, ±0.99 log10 reduction, Prontosan® solution), Pyridine (1.82, ±1 log10 reduction, Octenidine® solution) and Hypochlorous Acid (0.67 log10 reductions). The total mean ARRIVE score was nine out of twenty (±3.27, range 5–14). (S3)

Human studies analysis

A total of three human studies ($n = 3$ of 43, 7%) were identified which included fifty-four participants with DFU or VLUs. Only three topical agents were tested; iodine ointment (Cadexomer Iodosorb™),[51] a surfactant wound irrigation with melaleuca oil[48] (WoundAid®) and a PHMB surfactant wound irrigator (Prontosan®).[56] The studies were graded using the SIGN classification system[27] as level 2, grade of recommendation Level D. Two of the studies were a proof of concept methodology with small sample sizes, and one was a randomised controlled trial. All three studies did not incorporate any analysis past that of microbiological outcomes. All studies withheld topical antimicrobial or antibiotic treatment for at least one week prior to enrolment, obtained pre-and-post tissue specimens and used appropriate microscopy methods to confirm the presence of biofilm within wounds.

Borges et al. [56] employed a randomised control trial design to compare a one-off (one-minute exposure) application of either saline (control) or surfactant wound irrigation with PHMB in VLUs. Tissue punch biopsies were taken pre and post treatment to determine cfu/g tissue and the presence of biofilm was confirmed with TEM. TEM demonstrated bacterial biofilm in all wounds after treatment with both saline and PHMB. There was no reported difference in post-treatment cfu/g tissue between groups (saline = 3.77 log10 cfu/g tissue *versus* PHMB = 3.67 log10 cfu/g tissue).

Malone et al.[51] undertook a proof of concept design with seventeen participants to analyse the effect of cadexomer iodine applied to chronic DFUs every two days, for a total of seven days. The presence of biofilm was confirmed with SEM and PNA-FISH with CLSM, and any reductions in the total microbial loads were determined using qPCR of the 16S ribosomal rRNA gene. Most participants (11 of 18) achieved a 1 log10 or greater reduction in the total microbial load of DFUs (mean pre-treatment = 5.9 log10 16S copies/mg of tissue *versus* 4.5 log10 16S copies/mg of tissue).

Johani et al.[48] undertook an *in vitro* to *in vivo* study designed to examine clinically relevant exposure times of topical wound solutions in respect to chronic wound biofilm in DFUs. In the human *in vivo* study, ten participants had a topical agent (surfactant solution with melaleuca oil) applied daily for fifteen minutes. Pre-and post-tissue punch biopsies were obtained, and qPCR used to determine the total microbial load. The presence of biofilm was confirmed in all ten participants using SEM and PNA-FISH with CLSM. No change in total microbial load was detected after seven days of treatment (4.9 log10 16S copies/mg tissue *versus* 4.8 log10 16S copies/mg of tissue), and *P. aeruginosa* and *S. aureus* abundance increased with use.

Discussion

This review presents a detailed analysis of commercially available topical agents that have been tested for use in the management of chronic wound biofilms, encompassing *in vitro*, animal and human *in vivo* data. To the best of our knowledge, this is the first review of its kind. The scoring/grading systems utilised assist to determine the quality of the overall evidence, and collaterally serve to highlight the gaps within the current evidence.

Summary of key findings

In total, we identified 640 articles from our search criteria of which 531 failed to meet study inclusion criteria, or were duplicates. Of the 109 articles that proceeded to full review, sixty-six further articles were excluded (S3). This was due to; lack of appropriate visualisation techniques to confirm the presence of biofilm from human tissue samples, the use of the topical agent/s not in the context of chronic wounds or the topical agent/s were tested in a model designed for biofilm prevention, rather than eradication. Of the forty-three studies that were included in the final analysis, *in vitro* testing accounted for 90% ($n = 39$ of 43 studies) of all included studies, and only three (7%) eligible human studies were identified. This analysis clearly identifies a large disparity in the translation of laboratory studies to researchers undertaking human trials.

When analysing the thirty-nine included *in vitro* studies, a standardised methodological approach to biofilm testing was not observed. Sixteen different biofilm models were used with significant variations between test parameters such as; choices of

different bacterial strain or isolate ($n = 78$), biofilm growth time (24h to 168h), starting log densities, agent exposure duration (3s to 168h) and adaptions to *in vitro* models to more closely resemble a wound environment ($n = 22$ of 39, 56%). Thus, the significant variants identified in methodological designs limited the overall analysis and ability to compare the efficacy and outcomes between agents. In an attempt to circumvent the loss of data, the efficacy of topical agents *in vitro* on bacterial viability was analysed using the most widely reported quantification value (cfu), and by referencing the bacterial viability by exposure time endpoints for all agents (24-hours and total combined exposure times). As variations were consistently different across all studies, the results may not be truly indicative of an individual product, but can serve to demonstrate a trend in the overall data.

Gaps/limitations within the current body of research

In vitro

High throughput rapid screening *in vitro* models are an important tool in assessing the potential efficacy and safety of therapeutic drugs in many branches of medicine, and are often required before moving into animal testing.[72] Likewise, *in vitro* biofilm testing provides a useful screening tool to review a test agent's antibiofilm efficacy rapidly and with minimal cost.[18,73,74] However like any system, *in vitro* screening only has value if it is reproducible, has low user and situational variability, and most importantly has good predictive value. Unfortunately, as this review demonstrates, a standardised methodological approach of biofilm models for testing topical agents is not common, which leads to large disparities between testing conditions and limits meaningful analysis. A recent meta-analysis of antimicrobial efficacy against biofilms in *in vitro* studies found that the choice of experimental model and method has the greatest influence on outcome.[75] The bulk of studies included in this review reported a positive outcome for the agent they were testing, which raises questions about model choice and expected outcomes.

Two common themes of *in vitro* testing which are related, but not necessarily dependent issues are: (1) the robustness of standardised *in vitro* methods in terms of repeatability and reproducibility, and; (2) how well the *in vitro* method recapitulates *in vivo* conditions. The ultimate goal is to harmonise these two factors. A question still open for debate is are *in vitro* experiments so removed from real life conditions as to be irrelevant? [73] Conversely, if a product is shown to have poor efficacy *in vitro*, is there any merit in moving forward with a costly animal or clinical study?

Measure of efficacy

Do log reductions (reported as cfu/ml) for *in vitro* tests need to be more stringent to account for the variability that exists once a product or agent is tested in an animal model or beyond? Log reduction is a method that enables statistical evaluation of the data including determining sources of variability once the stakeholders agree upon a standard method for evaluating wound care products. The variability associated with the method can then be used to calculate the Type I and Type II error rates which are useful for regulatory decision making.[76]

A major limitation of log reduction as the measure of efficacy for medical products and devices is that it has no direct correlation to clinical settings. If translational data is the goal, then how is a clinician to interpret a log reduction value of 3, for instance, when treating a patient? As an industry there is a need to develop a better correlation coefficient, or perhaps a new interpretation of the log reduction that relates *in vitro* and clinical data in a more practical way. If the use of cfu/ml log reduction is adopted as the consensus measure, there should also be an established reference reduction amount. A six-log reduction has been suggested by the Environmental Protection Agency (EPA) as demonstrating good efficacy for high-level disinfectants against biofilms on hard non-porous surfaces with a CDC reactor model,[77–79] but no specific clinical guidelines for wound care products exists. However, there is also the argument that a topical agent should demonstrate complete eradication in a simple model such as MBEC, to ensure clinical efficacy in the more challenging setting of a human wound.[21] To further complicate the ability of researchers and clinicians to compare the efficacy of agents by a standardised method, some studies have only reported efficacy outcomes using alternate methods such as biofilm biomass[69] and optical density $_{600}$.[54,63,64,69] This makes comparison of agents impossible and supports the requirement of consensus regarding a standardised approach to reporting efficacy.

Bacteria

A plethora of different bacterial strains were identified in the included studies ($n = 78$). With this in mind, how do researchers decide on which bacterial strain/s to use? And which provides a closer relevance to microorganisms from human wounds? In general, the reporting of reasoning and selection of bacterial strains was poor. As an example, in the studies that used clinical bacterial strains, most did not perform testing to determine if it was a biofilm forming strain, nor were comparisons made with a similar laboratory strain. In some instances, bacterial strains were used from other areas of human disease, such as the laboratory strain *P. aeruginosa* (ATCC 9027) isolated from an outer ear infection,[31] or *S. aureus* (NCTCC 8325) isolated from a corneal ulcer.[29,41,58,69] The use of a validated reference strain would increase reproducibility and the ability to compare efficacy results. The bacterial inoculation quantity ranged from 10^5 to 10^8 cfu/ml, with some studies re-inoculating the model at intervals. The majority (87%, 34 of 39) of models used a single species design. This is not clinically representative of *in vivo* findings.[80–82]A starting inoculum should be of a meaningful log quantity to ensure an appropriate growth that is not immediately eradicated.[83–86]

The duration of bacterial growth of the studies included in this systematic review ranged from 24 to 168 h. Wolcott and colleagues[87] demonstrated that biofilms are more susceptible when immature, and that biofilm maturity is impacted by both species selection and model design. Phillips et al.[46] also demonstrated that cultures of *P. aeruginosa* or *S. aureus* required at least three days (72 h) of growth at 37 °C to develop biofilm communities that were tolerant to exposure to 0.5% bleach for ten-minutes or twenty-four hours of exposure to 50x MIC of gentamycin.[88] Conversely, Castenada et al.[49] demonstrated in orthopaedic biofilm infections, that a twenty-four-hour 96-well plate model might overestimate the amount of antimicrobial required, as the MBEC was lower when exposure time was increased.

Substrate

Biofilms grown on different substrates (*e.g.*, inert plastic or metal compared to biological substrates like collagen) usually develop significantly different sensitivities to antibiotics and antiseptics.[88,89] It is challenging to replicate the multi-cellular topography found clinically within an *in vivo* model[73], although researchers have created models and made alterations, such as the use of an *ex-vivo* porcine model[39,46,48,62,88] or through the addition of collagen.[66] This does not mean that data generated using inert synthetic substrates is irrelevant or useless, but readers and clinicians should be aware that the substrate on which a biofilm is grown can influence the effects of antibiotics and antiseptics on mature

biofilm communities. An *in vitro* biofilm is also characterised by attachment to a surface, a factor that is not representative of *in vivo* or *ex vivo* findings.[73]

The addition of wound exudate or flow to a model can increase the clinical relevance, however justification, reasoning and reporting should be clear. The addition of nutrients changes the way bacterial cells grow, and therefore the use of more representative media should be considered.[73] This review identified a variety of model modifications. A continuous tube flow reactor[90] was used in one study, a model which is not clinically representative or reflective of a chronic wound. CDC reactor or colony drip flow reactor models had varied exudate levels ranging from 11.7 ml/min[30] to 125 ml over 24 h[33], to 40 ml/h, which included negative pressure wound models.[45]

The strict inclusion and exclusion criteria applied to this review were considered necessary to ensure the clinical relevance. The decision to exclude *in vitro* biofilm prevention studies is supported by the general consensus that topical agents and antimicrobials should not be used prophylactically, and also supported by the knowledge that clinicians do not know which wounds may become chronic and complex, and potentially require antimicrobial use. Fourteen prevention only studies were identified and excluded, and nine of the included *in vitro* studies included both prevention and eradication models.[36,40,43,54,62,63,67-70] The strict biofilm visualisation inclusion criteria applied to human *in vivo* studies, excluded a potential eight studies that may have included wounds with biofilm.[91–98] While it is known that the majority of chronic wounds may be affected by biofilm,[12] if a company is claiming efficacy against biofilm specifically, the effect should be demonstrated and confirmed microscopically, (in addition to microbiological measures) as well as through clinical outcomes.

In summary, significant variation in *in vitro* biofilm testing of commercially available topical agents for use in wounds was identified. The modified MIABiE grading tool, although not validated, is reflective of the information required from a biofilm experiment to ensure it is good quality, and that it may be independently verified and interpreted. The low overall score of nine (±3.61) is representative of the quality of design and reporting, which reflects the aforementioned need for improvement.

This review and its authors advocate for a consensus or recommendation document that provides specific guidance. This should target researchers and companies who are involved in testing products against biofilm, as well as for consumers and clinicians to be able to more clearly appraise the evidence, and understand the results reported. The results of *in vitro* testing should not overstate the outcomes, and clinicians should remember that *in vitro* testing is far removed from clinical reality, and results are not directly translatable.

Animal

As demonstrated by the limited number of studies identified,[31,40,44,55,71] there are scant animal studies, and the identified studies had limitations in both reporting and study design. The ARRIVE guidelines[26] have been used as a grading tool for this review, and are supported by many journals as publishing requirements. This guideline and checklist primarily serves to guide researchers to produce good quality research and reporting, to maximise the availability and utility of the data gained from the experiment, and to prevent unnecessary animal wastage. For all agents tested, the combined average exposure time mean log10 reductions were lower than *in vitro* results (silver: animal = 0.6, ±0.45 *versus in vitro* = 2.82, ±2.13; iodine: animal = 4.5 *versus in vitro* = 5.05 ± 2.98; PHMB: animal = 2.37 ± 0.99 *versus in vitro* = 3.47 ± 2.42). This review identified some concerns regarding study design. Randomisation and allocation of treatment was not discussed in any of the studies, with only Davis and colleagues[55] avoiding treatment bias due to all animals receiving the same topical treatments. Gawande and colleagues[40] introduced the potential for bias through allocation of treatment following bacterial inoculation and the growth period. A sample size or power calculation was not discussed for any of the included studies, nor was progression to human testing or generalisability of the results to human biology reported. Only Davis and colleagues[55] reported on the choice and reasoning in selection of the animal model.

Human

The small number of human *in vivo* studies ($n = 3$) which included a total of fifty-four participants demonstrates the paucity of clinically relevant biofilm research. Perhaps most importantly, it highlights that practically no commercially available topical agents for use in human wounds have evidence for efficacy against wound biofilms. Malone et al. [51] identified that cadexomer iodine resulted in a one – two log10 reduction in the total microbial load of wounds complicated by biofilm, whereas both Johani and colleagues,[48] and Borges and colleagues[56] identified that surfactant solutions of melaleuca oil or PHMB had little effect on the total microbial load of wounds complicated by biofilm. The three studies self-reported proof of concept study designs and therefore were not clinically representative of the typical duration a topical agent can be used for, and did not incorporate other management strategies for wounds, such as sharp debridement.[48,51,56] A Global Wound Biofilm Consensus[18] recommends an aggressive step-down approach to managing chronic wound biofilm, which includes the use of topical agents, which is not feasible to investigate in a pilot or single intervention study.

Limitations of the review

The major limitation of performing this review was the heterogeneity of data which was compounded by the inclusion of three systems (*in vitro*, animal and human *in vivo* studies). This prohibited meta-analysis of the results, which reduced the strength of the evidence to support any particular product. More than half of the reviewed papers (65%, 28 of 43) were funded directly by medical companies, or undertaken by researchers employed by the product company, and a further five (12%, 5 of 42) did not comment on conflict of interest or funding source. This is crucial in considering the confidence in results, as there is no assurance for clinicians that the woundcare company has not simply manipulated and stipulated the test parameters to ensure optimal results for their product. The fact that three quarters of the identified studies have a risk of publication bias or may be influenced by favourable reporting limits the confidence in the outcome, and supports a requirement for corroboration by independent laboratory testing.

The review identifies that the majority of evidence available is *in vitro* (90%), with low quality, and high variability. This finding supports increased standardisation of aspects of *in vitro* models, including clear and standardised reporting of design and results, appropriate bacteria growth durations and clinically relevant agent exposure periods. While *in vitro* testing can improve, the knowledge remains that bacterial transcriptome is different between *in vitro*, animal, and human chronic wounds, and therefore designed models are not truly reflective of the clinical processes.[72]

The surprising lack of *in vivo* human studies can be interpreted as a clearly identified requirement for further research in general, and more specifically, clinically representative treatment periods.

In summary, there is not sufficient evidence to definitively recommend one product over another, and the heterogeneity of study designs limits any outcome suggestion other than the effect on microbial load, which thus far has not been demonstrated to have an effect on wound healing.

Declaration of Competing Interest

None.

Acknowledgments

Raymond Robinson from Ulster University for reviewing the manuscript.

Funding

This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

Transparency declaration

MM, PS, GS, TB, GJ received a consultancy fee from S&N to undertake work not associated with this study but pertaining to producing educational material on biofilms in chronic wounds. MM received a research grant from Smith and Nephew and Mundi Pharmaceuticals for studies included in this review. All other authors have nothing to declare

Supplementary material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2019.12.017.

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