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Antimicrobial dressings: Comparison of the ability of a panel of dressings to prevent biofilm formation by key burn wound pathogens

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ABSTRACT

Antimicrobial medicated dressings (AMD) are often used to reduce bacterial infection of burns and other wounds. However~~, since AMD are medical devices~~, there is limited literature regarding comparative efficacies to inform effective clinical decision making.

Objectives

Following on from a previous study where we demonstrated good antibiofilm properties of acetic acid (AA), we assessed and compared the *in vitro* anti-biofilm activity of a range of AMDs and non-AMDs to AA.

Methods

Laboratory experiments ~~were used to determine~~determined the ability of a range of eleven commercial AMD, two nAMD, and AA, to prevent the formation of biofilms of a panel of four isolates of *Pseudomonas aeruginosa* and *Acinetobacter baumannii*.

Results

There is a large variation in ability of different dressings to inhibit biofilm formation, seen ~~both with~~between dressings that contain the same, and those that contain ~~a variety of~~ other antimicrobial agents. The best performing AMD were Mepilex® Ag and Acticoat. AA consistently prevented biofilm formation.

Conclusions

~~Vast~~Large variation exists in the ability of AMD to prevent biofilm formation and colonisation of wounds. A standardised *in vitro* methodology should be developed for external parties to examine and compare the efficacies of commercially available AMDs, along with robust clinical randomised

controlled trials. This is essential for informed clinical decision-making and optimal patient management.

Keywords: Antimicrobial, dressings, wounds, burns, biofilms.

1 INTRODUCTION[1]

Infection is a significant concern in patients who survive an initial burn insult. This complication of burn recovery impacts on morbidity, mortality and healthcare costs [1], and in some centres has been estimated to account for over 75% of the mortality [2].

Burns patients are especially susceptible to infection owing to the injury removing the protective barrier provided by the skin, combined with general immunosuppression, the presence of endogenous microflora, prolonged hospital stays, and invasive diagnostic and therapeutic procedures [3]. Consequently despite careful treatment and infection control practices, burn wounds are readily colonised with a range of pathogenic micro-organisms, significantly delaying wound healing, and increasing risks of systemic infection, and graft failure [4].

The most frequently implicated bacteria are *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Enterobacter* spp [5, 6]. Of these, *P. aeruginosa* and *A. baumannii* are most prevalent [7], with Lawrence [8] finding *P. aeruginosa* in one-third of burn wounds, and in 59% of those patients with extensive burns. Yali *et al* [9] took clinical samples from burns patients in burn intensive care units (ICU) and common burn wards and identified the organisms causing infection. 1621 pathogens were isolated from 2395 clinical samples of the burn ICU, and of these 74.2% were Gram-negative. *A. baumannii* was the most prevalent representing 34.4% of all pathogens present in this setting. Additionally, there is also concern that patients may acquire bacteria with resistance to multiple systemic antimicrobials, such as the carbapenem resistant *Enterobacteriaceae* (CRE), for which there are very limited treatment options.

Colonisation of burn wounds typically occurs as biofilms (communities of bacteria), which are harder to treat and eradicate owing to reduced rates of metabolism and protection (against antimicrobial

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[1] AMD: antimicrobial dressing; nAMD: non-antimicrobial dressing; AA: acetic acid; ICU: intensive care unit; AM: antimicrobial; RCTs: randomised controlled trials; MH: muller-hinton; CV: crystal violet.

agents and the immune response) afforded by the polysaccharide matrix [10]. Consequently the presence of biofilms is associated with persistence of colonisation and increased risk of systemic infection [1]. Hence, general principles of wound management include appropriate systemic care (e.g. in terms of pain control, nutrition and control of serum glucose levels in those with diabetes mellitus), combined with local wound care (especially in terms of preventing colonisation). For burn wounds, the standard of care worldwide is early excision of necrotic tissues followed by covering the wound with a medical dressing. Prevention and treatment of bacterial colonisation are key parts of wound care [11].

There is a ~~vast~~large array of dressings and a range of factors that govern the choice of dressing that is most appropriate for wound management (e.g. type of wound, stage of healing process, and volume of exudate). However, for burns and other wounds where infection is a high risk, antimicrobial dressings (AMD) may be used. Typically the antimicrobial agent (AM) is contained within a commercially marketed wound dressing, which can be used both prophylactically (to prevent colonisation of the wound and subsequent biofilm formation), and in the treatment of established infection. Systemic administration of antimicrobials is not thought to be necessary nor useful for the management of local wound infections, since the drugs i) may not penetrate well into the wounds (due to poor blood flow and the presence of dead tissue) [10], ii) would need to be used in very high doses (to treat organisms growing in sessile biofilms) [12], and iii) systemic administration has not been shown to prevent bacterial colonisation [13]. Furthermore, inappropriate use of systemic antibiotics can be associated with problems of allergy, toxicity and the development of resistance in non-target organisms.

AMD account for approximately a quarter of all dressings prescribed in primary care in England [14], and may contain a range of antimicrobial agents (e.g. silver, iodine, honey, and chlorhexidine). The use of AMD and silver-dressings (which are classed as ‘advanced’ dressings) has risen in recent years,

with £25 million spent on silver dressings in 2006/7 [10]. Indeed, one in every seven wound dressing items prescribed by the NHS contain silver as an active agent [10].

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Silver (Ag) has been used extensively in burn wound management [15] and is a potent antimicrobial. Silver-containing dressings vary in their composition and act by a combination of i) absorbing wound exudates and killing the microorganisms drawn into the dressings, and/or ii) releasing active silver onto the wound bed. These biologically active ions then bind to negatively charged proteins, RNA, and DNA and damage bacterial cell walls, inhibit replication and reduce metabolism and growth [16]. Broad antimicrobial activity has been reported against Gram-positive and Gram-negative organisms [17], protozoa, viruses [18], and fungi [19].

AMD are marketed as effective against a broad range of bacteria (growing as biofilms) over multiple days, and are indicated for a variety of serious wounds (e.g. partial thickness burns, ulcers, donor and graft sites, traumatic, and surgical wounds). Provided that the agent is considered to only provide an ancillary action on the wound, the majority of dressings (including AMD) are classified as medical devices [20]. This means there are lesser requirements in terms of robust data from randomised controlled trials (RCTs) to support safety and efficacy, and literature reviews and commercial company-led research are often deemed acceptable for licensing. Consequently, there is little data available in peer-reviewed literature concerning their activity [11]. Unsurprisingly in clinical practice, opinions on the use of silver dressings are divided, with some clinicians believing that they have a role to play in preventing infection in burns patients [21, 22, 23], and other experts not endorsing their use owing to a lack of evidence of effectiveness [10, 24].

Several systematic reviews have been performed looking at use of silver dressings for wound management with the majority concluding that there is insufficient evidence to recommend using silver dressings. A systematic review performed by [25] identified 14 RCTs of silver-containing dressings and topical silver agents (used with dressings) for burn wounds, and despite significantly better healing with silver compared to the control in one small trial, they concluded that silver-

containing dressings were either no better, or were worse than control dressings in preventing wound infection. Indeed, a Cochrane Review from 2010 looking at topical silver products (dressings and creams) identified 26 RCTs (20 of which were on burns), and concluded that there was 'insufficient evidence to support the use of silver containing dressings or creams, as generally they did not promote wound healing or prevent wound infections' [26]. However, despite these findings, clinicians are still using silver dressings perhaps owing to the extensive marketing and promotion of these commercial dressings [27], and the absence of any alternatives.

In addition to silver, AMD may contain iodine/povidone-iodine (which rapidly penetrates microorganisms, damaging proteins, nucleotides and fatty acids, leading to rapid cell death) [28], honey (which is antimicrobial due to osmotic effect, a low pH and the production of hydrogen peroxide [29]), or chlorhexidine; which binds to and disrupts the negatively charged bacterial cell wall and affects the osmotic equilibrium of the cell [30]. Furthermore, in addition to commercial AMD, ~~biocides~~other biocide-impregnated dressings may have a role to play in preventing wound infection. A range of biocides have been investigated in this regard (e.g. silver nitrate, mafenide acetate, povidine iodine, silver sulfadiazine and chlorhexidine), including acetic acid (CH_3COOH).

Acetic acid (AA), or vinegar, has been used sporadically in medicine for the past 6000 years [4], being successfully implemented to treat plague, ear, chest, and urinary tract infections [31, 32, 33], and in the elimination of *Bacillus pyocyaneus* (now *Pseudomonas aeruginosa*) from war wounds [7]. We have used AA for a decade in our burns centre at a concentration of 2.5% to treat patients with burn wounds infected or heavily colonised with *P. aeruginosa*. Here it is applied topically within dressings, is well-tolerated by patients, and is observed to have good clinical outcomes. Additionally, AA is currently used in a number of lesser economically developed countries (LEDCs) and other resource-limited settings for burn wound management.

Following a recent study on the anti-biofilm properties of AA as a topical AM agent, we sought to assess and compare the anti-biofilm properties of AA versus the AMDs currently used in our Burns Centre at the Queen Elizabeth Hospital, Birmingham.

We aimed to compare efficacy to help guide clinical practice at our centre and others.

2 METHODS

A series of *in vitro* experiments were conducted to determine the efficacy of the AMD in terms of their ability to prevent biofilm formation. AA (in a range of concentrations from 5% down to 0.02%) was included as a comparator following on from previous research [Halstead *et al*, unpublished] which demonstrated AA to be effective at preventing biofilm formation when used from 5% down to concentrations as low as 0.31% (w/v). Plain dressings that contained no antimicrobial agent (herein referred to as nAMD) were also included as comparators.

Four organisms were tested (two *Pseudomonas aeruginosa* and two *Acinetobacter baumannii*) (Table I), and comprised well-characterised control strains (PS_PA01, ACI_AYE) and clinical isolates from burns patients (ACI_721, PS_1586). All AM products (Table II) were freshly opened and were within date when used. Experiments were performed using at least two biological replicates, and at least four technical replicates of each isolate.

2.1 Processing of the AMDs

The following AMD were prepared for testing: Mepilex® Ag (Mölnlycke Healthcare), Aquacel® Ag, Aquacel® Ag Foam, Aquacel® Ag Burn (all Convatec), UrgoTul® Silver (Urgo Medical), Acticoat (Smith & Nephew), PolyMem Silver® (Ferris MFG. Corp.), Inadine® (Systagenix), L-Mesitran® Net, L-Mesitran® Hydro (both from L-Mesitran Wound Care), and Bactigras (Smith & Nephew). This involved carefully

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cutting the sterile dressing into a number of 1cm^2 pieces (so that there was sufficient for 1 piece per test well) using a sterile scalpel or a pair of flame sterilised scissors. The nAMD; UrgoTul® (Urgo Medical), and PolyMem® (Ferris MFG. Corp.), were prepared in the same way.

Details of these dressings (and references to published work) are in Table II.

2.2 Impact of the AMD and AA on biofilm formation

The ability of the range of AMD and AA to prevent biofilm formation was assessed using a crystal violet biofilm formation assay as described by Baugh *et al* [34].

Overnight cultures of the test strains (grown in 5ml of Lysogeny (LB) broth [Oxoid]) were diluted in fresh antibiotic-free Muller-Hinton (MH) broth [Oxoid] to an optical density at 600nm (OD_{600}) of 0.1, and then 1ml was seeded into wells of a 24-well MTT [Corning, New York], alongside 1ml of either diluted AA (water as diluent) or sterile water. AA was tested at the following dilutions: 5%, 2.5%, 1.25%, 0.63%, 0.31%, 0.16%, 0.08%, 0.04%, 0.02% and 0.01%. For the AMD test wells, one piece of dressing was placed into the well containing the organism suspension and water to provide a total volume of 2mls plus dressing.

Suitable controls were included in each assay, comprising 1ml overnight bacterial culture with 1ml water (for the positive control), or 2mls MH broth with no bacteria (for the negative control).

Plates were sealed and statically incubated at (33°C); the temperature of the surface of a wound [35]. After 72 hours, the liquid and AMD pieces were removed from the wells and the plates rinsed in tap water to remove any unbound cells. Any existing biofilms were then visualised through staining with 2mls of 1% crystal violet (CV) [Sigma Aldrich, Poole, UK], further rinsed (as above) to remove unbound CV, and dye solubilised by the addition of 2mls of 70% ethanol. 200ul from each well was then transferred into wells of a 96-well microtitre tray, and the OD_{600} of the solubilised CV

solution was then measured using a FLUOstar Optima [BMG Labtech] to assess the biomass of the biofilms.

The positive and negative controls for each test plate were examined and if within a normal range the rest of the data was analysed for percentage change in biofilm biomass, and for statistical significance, by comparing values for each AMD, and at each concentration of AA to untreated (positive) controls using the ~~students' 't' test.~~Students' 't' test. Adjustments for multiple comparisons were made to control the family-wise error rate for each of the four groups of tests using Holm's method [36].

3 RESULTS

All four of the bacterial isolates (PS_PA01, PS_1586, ACI_AYE and ACI_721) were tested against all the AMD, nAMD and AA achieving at least four, but up to ten technical replicates. The ~~number~~numbers of replicates ~~can be seen in parenthesis in table~~are shown on tables III~~.~~ and IV for *P. aeruginosa*, and *A. baumannii*, respectively.

The mean average optical densities of the solubilised CV were plotted per species for *A. baumanniii*, and *P. aeruginosa*, and are shown in figures 1 and 2, respectively for the dressings, and figures 3 and 4 for the AA and best/worst performing dressings against each species, respectively. The standard error bars (denoting variation in the number of technical replicates) are also plotted and all data has been normalised by subtraction of the negative (broth only) control.

The graphs demonstrate that there is a large variation in the test agents (AMD, nAMD and AA) in terms of reducing biofilm formation (e.g. from an increase of 33% with L-Mesitran® Net to a decrease of 100% with Acticoat and Mepilex® Ag for ACI_721). This is seen ~~both with~~between different dressings that contain the same active agent (e.g silver) (e.g. from an increase of 43% with PolyMem Silver® to a decrease of 100% with Acticoat and Mepilex® Ag for PS_PA01) and between those that

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contain ~~a variety of other~~different AM agents. This data is also shown in table III which provides percentage differences in biofilm biomass, and statistical significance ($p \leq 0.05$) when the difference in biofilm biomass for each dressing/agent was compared to the positive control. The p-values in tables III and IV are adjusted for multiple comparisons to control the error rate.

Generally all AMD showed similar activity against both representatives of each species.

3.1 Performance of the silver-containing AMD

Seven silver-containing AMDs were tested and the majority showed some effectiveness at reducing biofilm formation (~~table~~tables III & IV, figures 1 & 2). For both species and all four isolates, Mepilex® Ag (Mölnlycke Healthcare), and Acticoat (Smith & Nephew) were highly effective, leading to 90-100% reduction of biofilm formation compared to the positive control. These results were highly consistent across all replicates as shown by the small error bars, and were also statistically significant in the t-tests with all p-values ≤0.05.

For the Aquacel® dressings (Ag, Ag foam and Ag burn), the reductions were generally modest, averaging 44% for PS_PA01 and 34% for PS_1586. The *A. baumannii* isolates appear to be more susceptible to these dressings with average reductions of 77% for ACI_721, and 65% for ACI_AYE. The results for Aquacel® Ag burn against biofilms of ACI_AYE show reductions of 94% (statistically significant with adjusted p-values <0.05), and small standard error across all six replicates.

In general UrgoTul® Silver (a thin mesh-like AMD) was able to reduce biofilm formation for the majority of the isolates and replicates. However, for PS_1586, for four of the eight replicates, there was an increase in biofilm biomass in wells containing the dressings. This ranged from 13-80% (data not shown). PolyMem Silver® also gave varied results, with reductions in biomass of biofilms apparent with the *A. baumannii* isolates (61% reduction for ACI_AYE and 75% for ACI_721), but increases noted with both the *P. aeruginosa* isolates (43% increase in biofilm biomass for PS_PA01, and 11% increase for PS_1586).

3.2 Performance of the non-silver containing AMD

The four non-silver containing AMD gave varied results. Inadine® (Systagenix), which contains povidone-iodine as the active agent, slightly reduced biofilm formation for the two clinical isolates (ACI_721 and PS_1586 ~~and ACI_721~~) by 6 and 10% respectively, but this was not statistically significant, and the dressing was ineffective against the control strains.

The honey-containing dressings of L-Mesitran® Net and L-Mesitran® Hydro (both from L-Mesitran Wound Care) were generally ineffective at preventing biofilm formation. Although reduced biofilm formation occurred with *A. baumannii* ACI_AYE for both dressings with a maximum reduction of 10.4% (not statistically significant, p values >0.05), and ACI_721 (where there was a statistically significant reduction (adjusted p = 0.~~004~~038) of 62% in biofilm biomass with L-Mesitran® Hydro compared to the positive control), both dressings were ineffective at preventing biofilm formation of *P. aeruginosa.* For both isolates increased biofilm formation occurred, ranging from 20% with L-Mesitran® Net and PA_1586 to 200% with L-Mesitran® Hydro and this same isolate. L-Mesitran® Hydro was the worst performer, with an average 115% increase in biofilm biomass for PS_PA01, and average 200% increase for PS_1586 (Table III).

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Bactigras (the only chlorhexidine-containing dressing) generally reduced biofilm formation, with ~~statistically significant~~ reductions of 39, 59 and 68% for PS_PA01, ACI_AYE and ACI_721, respectively~~.~~ (with this latter reduction statistically significant with an adjusted p-value of 0.038). It was however ineffective for PS_1586, where there was an average 200% increase in biofilm biomass.

3.3 Performance of the AMD vs the nAMD

Despite not containing an AM agent, both of the nAMD reduced biofilm formation in this experiment for the *P. aeruginosa* (PS_1586), and *A. baumannii* (ACI_AYE and ACI_721) isolates. Reductions

ranged from 7% to 74%, but were ~~generally~~only statistically significant for ACI_721, where the 74% reduction is associated with an adjusted p-value of 0.003 (table ~~III) and~~IV). Some of the reductions were higher than those seen with some of the marketed AMDs. For example for ACI_~~AYE~~721, Inadine®, L-Mesitran® Net and L-Mesitran® Hydro resulted in differences of ~~+3, -10.4~~ 6, +33, and -~~1~~62%, compared to the nAMD (PolyMem® plain) where there was a ~~66~~74% reduction in biofilm biomass (statistically significant, p=0.003).

For PS_PA01, there was no reduction in biofilm biomass with the nAMD (table III).

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3.4 Performance of the AMD compared to AA

The shaded cells in ~~table~~tables III and IV highlight the data where there was reduction in biofilm formation by at least 90%. Acetic acid performs well as an anti-biofilming agent, with reductions of ≥90% seen for concentrations of AA from 5% down to 0.16% (0.08% for ACI_721). This result was consistent across all replicates.

Graphs 3 and 4 show the optical density of the biofilm biomass produced following incubation of the cultures with the various concentrations of AA alongside the most and least effective of the AMDs (Acticoat and L-Mesitran® Net for *A. baumannii*, and Mepilex® Ag and L-Mesitran® Hydro for *P. aeruginosa*).

The data demonstrate that AA out performs the L-Mesitran® dressings in terms of reducing biofilm formation, and compares favourably to the best-performing AMD (Acticoat and Mepilex® Ag).

4 DISCUSSION

Medicated AMDs have the potential to significantly reduce bacterial contamination of burns and wounds; a post-insult complication that may delay wound healing, and lead to widespread systemic infection [~~36~~37]. Despite being a small study, this work has demonstrated that in the *in vitro* setting

there is a large variation in the ability of commercial AMD to prevent biofilm formation of two key burn wound pathogens. Biofilm formation is a key contributor to wound colonisation and subsequent infection.

Although not concerning biofilms, Cavanagh *et al* [11] found similar results when they tested the antimicrobial efficacy of a range of silver dressings (Mepilex® Ag, Algicell™ Ag, PolyMem Silver®, Biostep™ Ag, and Acticoat) against planktonic forms of growth. In a log-reduction assay (from Gallant-Behm *et al* [~~37~~38]), they determined the ability of commercial silver AMD to kill *Staphylococcus aureus* in 30 minutes. They noted a large variation in average log reduction between the silver dressings and concluded that Acticoat was the only bactericidal dressing.

Here we show that the silver dressings were the most effective at preventing biofilm formation, with Mepilex® Ag and Acticoat consistently outperforming the other AMD and reducing biofilm formation by at least 90%. A review of the literature shows that many comparisons of silver dressings have drawn similar conclusions regarding efficacy of Acticoat. For example Gallant-Behm *et al* [~~37~~38] found that Acticoat was the only bactericidal dressing of eight that were tested.

Lesser reductions in biofilm formation were seen with the other silver-containing AMD, although the results were still mostly statistically significant when compared to the positive control. The worst performing AMDs were the honey-containing dressings, where there was little, if any, observable benefit over the two nAMD. Additionally, the study has provided further evidence that AA can prevent the formation of biofilms by key burn wound pathogens, and has indicated that this activity compares favourably to the best performing AMDs. The antimicrobial effect of AA against planktonic and biofilm growth modes of bacteria has been reported previously [~~38,~~ 39, 40], but comparison to AMD is a new finding.

~~To further build up this evidence base~~In future, it would be useful to perform a number of ~~-~~ well controlled studies that take into consideration the exact dressing volume and quantities of AM agent

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316 that are released. In this study dressings were measured using a ruler and cut to 1cm^2, but their
317 volumes were not considered. A number of the dressings are thick foam (Mepilex® Ag, Aquacel® Ag
318 Foam, PolyMem Silver® and PolyMem®), and hence the tested volume of these dressings would have
319 been considerably more than that of the thinner dressings (the remainder of the panel). Additionally
320 the dressings were used in the experiment as they would have been in the clinical setting and
321 therefore, no allowance was made for the quantity of the AM agent released from the dressings, nor
322 the site or mode of release. Cavanagh et al [11] performed a silver-dissolution assay and report the
323 24-hour silver release for Mepilex® Ag and Acticoat as 0.698 and 0.144 mg/cm^2, respectively,
324 compared to 0.00014 mg/cm^2 for PolyMem Silver®. Our findings suggest that the amount of
325 released silver could be an important determinant of anti-biofilming activity and therefore future
326 studies should be done to measure the silver release from the dressings throughout the course of
327 the 72 hour experiment.
328
329 Although an *in vitro* experiment is unlikely to mimic biofilm formation in the *in vivo* setting, the
330 experimental model used was most appropriate for testing the dressings based on the release of the
331 active antimicrobial agent into 'exudate'. Additional experiments should be performed to assess
332 antibiofilm properties of dressings that rely on contact with a solid surface for release of the
333 antimicrobial agent, and should also test a larger panel of Gram-negative organisms as well as some
334 Gram-positives organisms such as *S. aureus* and *Enterococcus* spp. Furthermore, experiments should
335 also be conducted on pre-formed biofilms to test efficacy of the AMD and AA against established
336 bacterial colonisation of burn wounds.
337
338 It should be remembered that there are many factors that govern the choice of dressing, and indeed
339 the choice of AMD. Although important, bacterial load reductions are only one aspect of wound
340 healing, and therefore despite showing that certain dressings are better than others for bacterial
341 reduction, this is only one consideration for a clinician choosing a dressing.

5 CONCLUSIONS

The NHS spends a large amount of an ever-pressured budget on commercial AMD, and especially those that contain silver. This is despite a range of publications and systematic reviews concluding that there is no robust evidence that dressings containing AM agents (silver, iodine or honey) are more effective than unmedicated dressings for the prevention or treatment of wound infection [10].

This work has shown that there is a ~~vast~~large variation in the ability of commercial AMD to prevent biofilm formation and therefore colonisation of wounds, and that a number of the AMD are not able to prevent biofilm formation and are no better than the nAMD. We have also shown that AA consistently prevents biofilm formation for all isolates with low error bars and lower cost than the AMD (data not included).

Given their classification as medical devices, and the subsequent paucity of reliable and unbiased data on their effectiveness, a standardised *in vitro* methodology should be developed in order for external parties to examine and compare the efficacies of the commercial AMDs, along with robust clinical randomised controlled trials. These are essential for informed clinical decision-making and optimal patient management.

Clinicians should be wary of the use of AMDs (if intended to prevent or treat infections) in the absence of data showing anti-biofilm efficacy, since the longer a biofilm is present, the greater potential there is for systemic infection to occur.

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7 Transparency declarations

None to declare.

8 Disclaimer

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Table I: List of the control and clinical isolates used in this study

Study Identifier	Organism	Description
PS_PA01	*Pseudomonas aeruginosa*	Control strain [ATCC_15692]. Originally isolated from an infected wound.
PS_1586	*Pseudomonas aeruginosa*	QEHB Clinical burn wound isolate.
ACI_AYE	*Acinetobacter baumannii*	Control strain [ATCC® BAA-1710]. Originally isolated from human blood.
ACI_721	*Acinetobacter baumannii*	QEHB Clinical burn wound isolate.

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~~Table II:~~ ~~List of the dressings/agents used in this study alongside their supplier, antimicrobial agent and formulation, and reports/references on their activity~~

Dressing [Agent]	Supplier	Antimicrobial agent and formulation	Reports/References
Mepilex® Ag [Silver]	Mölnlycke Healthcare	Silver sulphate (Ag_2SO_4) dressing. Thick, soft silicone foam dressing	Dressing inactivates a wide range of bacteria within 30 minutes, provides a rapid and sustained silver release, can be worn for 7 days and does not stain [~~40~~41]
Aquacel® Ag [Silver]	Convatec	Ionic silver impregnated hydrofibre pad composed of sodium carboxymethylcellulose and 1.2% ionic silver	AQUACEL® Ag Foam dressings contain ionic silver to kill a wide variety of micro-organisms, (including certain tested antibiotic-resistant bacteria) within 30 minutes, and provide sustained bacterial killing for up to seven days~~. [41~~ [42]
Aquacel® Ag Foam [Silver]	Convatec	As above	As above
Aquacel® Ag Burn [Silver]	Convatec	As above	As above
UrgoTul® Silver [Silver]	Urgo Medical	Hydrocolloid dressing consisting of a polyester web, impregnated with carboxylmethyl cellulose, Vaseline and silver.	Many reports [See JWC educational supplement]. An example is 102 patients with critically colonised venous leg ulcers who were treated with Urgotul Silver versus plain Urgotul. After 8 weeks, there was a significantly greater reduction in wound size in the Urgotul Silver group ($p=0.002$) as well as fewer clinical signs of critical colonisation ($p<0.001$).
Acticoat [Silver]	Smith & Nephew	Nanocrystalline silver impregnated pad consisting of three layers	Dressing kills bacteria *in vitro* in 30 minutes, acts as an antibacterial barrier for up to 3 days, provides sustained silver release, and is effective against over 150 microorganisms (Gram-positive, Gram-negative, yeasts and molds) [~~42~~43].
PolyMem Silver® [Silver]	Ferris MFG. Corp.	Polyurethane membrane matrix containing F68 surfactant, glycerol, a starch copolymer and silver.	
Inadine® [Iodine]	Systagenix	Low adherent knitted viscose fabric impregnated with a polyethylene glycol (PEG) base containing 10% povidone iodine (combination of polyvinylpyrrolidone and elemental iodine).	Broad spectrum of activity against Gram-positive and Gram-negative bacteria, anaerobes, yeast, fungi and spores [~~43~~44].

Dressing [Agent]	Supplier	Antimicrobial agent and formulation	Reports/References
L-Mesitran® Net [Honey]	L-Mesitran Wound Care	Non-adherent open polyester mesh coated with a thin layer of L-Mesitran® Hydro gel.	L-Mesitran is a broad-~~spectrumantimicrobial~~spectrum antimicrobial, effective against most bacteria including MRSA and VRE [~~44~~45]
L-Mesitran® Hydro [Honey]	L-Mesitran Wound Care	Hydrogel sheet (1mm thick) attached to a semi polyurethane membrane by a thin fibrous bonding layer. The hydrogel contains 30% of medical grade honey.	As above
Bactigras [Chlorhexidine]	Smith & Nephew	Chlorhexidine Acetate BP 0.5% in white soft paraffin BP.	Bacteriostatic and bactericidal. Chlorhexidine acetate has been shown to be active, in vitro, against a wide range of Gram-positive and Gram-negative bacteria at concentrations of 10-50 µg/ml. These include: Streptococcus pyogenes, Enterococcus faecalis, Corynebacterium diphtheriae, *Strep. pneumoniae, S. aureus, Proteus vulgaris, E. coli,* and *P. aeruginosa* [~~45~~46]
UrgoTul® plain [No AM agent]	Urgo Medical	n/a	n/a
PolyMem® plain [No AM agent]	Ferris MFG. Corp.	n/a	n/a
Acetic acid (5% stock)	Tayside Pharmaceuticals	Acetic acid (CH_3COOH)	Bactericidal and active against biofilms when used at low concentration for a range of important burn wound pathogens [~~38,~~39, 40].

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Table III: Table showing the percentage (%) change in biofilm biomass for each of the isolates when coincubated with each of the AMD, nAMD or AA for 72 hours, when compared to an untreated, positive control.

	P. aeruginosa							*A. baumannii*					
Mepilex® Ag	-100 (8)*	8	<0.001	0.001	Yes			-99.9 (6)*	-95.9 (8)*	-100 (6)*	<0.001	0.001	Yes
Aquacel® Ag	-49 (8)*	change in biofilm biomass^Δ	p-value^	p-value*	significance•			change^Δ	-35 (6)	p-value^	p-value*	significance•	
Aquacel® Ag Foam	-21 (8)*	8	0.064	0.635	-			-36 (6)*	-66 (8)*	-74 (6)*	0.005	0.078	
Aquacel® Ag Burn	-63 (6)*	6	0.020	0.219	-			-31 (4)	-94 (6)*	-82 (4)*	0.601	1.000	
UrgoTul® Silver	-47 (10)*	10	0.002	0.029	Yes			+16 (8)	-20 (10)	-4 (8)	0.457	1.000	
Acticoat	-100 (8)*	8	<0.001	0.001	Yes			-94 (6)*	-96 (8)*	-100 (6)*	<0.001	0.005	Yes
PolyMem Silver®	+43 (8)	8	0.432	1.000	-			+11 (6)	-61 (8)*	-75 (6)*	0.521	1.000	
Inadine®	+3 (8)	8	0.488	1.000	-			-10 (6)	+3 (8)	-6 (6)	0.609	1.000	
L-Mesitran® Net	+38 (6)	6	0.808	1.000	-			+20 (4)		-10.4 (6)	+33 (4)	1.000	
											0.550		
L-Mesitran® Hydro	+115 (8)	8	0.148	1.000	-			+200 (6)		6	0.316	-1 (8)	-62
												.000	
Bactigras	-39 (8)*	8	0.005	0.070	-			+200 (6)		-59 (8)*	-68 (6)*	1.000	
										6	0.292		
UrgoTul® plain	+56 (4)	4	0.139	1.000	-			-17 (4)*	-7 (6)	-27 (4)	0.051	0.660	
PolyMem®	+39 (6)	6	0.665	1.000	-			-27 (4)*	-66 (6)*	-74	0.055	0.660	

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	plain				
~~Acetic acid 2.5%~~	~~-90 (8)*~~	~~-91 (8)*~~	~~-93 (10)*~~	~~-92 (8)*~~
~~Acetic acid 1.25%~~	~~-94 (8)*~~	~~-90 (8)*~~	~~-93 (10)*~~	~~-93 (8)*~~
~~Acetic acid 0.63%~~	~~-94 (8)*~~	~~-94 (8)*~~	~~-93 (10)*~~	~~-96 (8)*~~
~~Acetic acid 0.31%~~	~~-95 (8)*~~	~~-97 (8)*~~	~~-93 (10)*~~	~~-96 (8)*~~
~~Acetic acid 0.16%~~	~~-96 (8)*~~	~~-86 (8)*~~	~~-90 (10)*~~	~~-94 (8)*~~
~~Acetic acid 0.08%~~	~~-64 (8)*~~	~~-23 (8)*~~	~~-28 (10)~~	~~-95 (8)*~~
~~Acetic acid 0.04%~~	~~+35 (8)~~	~~+5 (8)~~	~~+10 (10)~~	~~+5 (8)~~
~~Acetic acid 0.02%~~	~~+42 (8)~~	~~-7 (8)~~	~~+6 (10)~~	~~+13 (8)~~
~~Acetic acid 0.01%~~	~~+67 (6)~~	~~+11 (4)~~	~~-30 (4)~~	~~+7 (4)~~

~~**Table III footnote:** + and – refer to increases and decreases in biomass, respectively, with the of replicates shown in parenthesis. Asterisks (*) denote statistically significant changes in bio (when compared to the positive control), and shaded cells~~

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Table III: Table showing the percentage (%) change in biofilm biomass for each of the *Pseudom* nAMD or AA for 72 hours, when compared to an untreated, positive control.

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Dressing/agent	*P. aeruginosa* PA01					*P. aeruginosa* PA_1586				
	Percentage change$^{\Delta}$	Number of replicates	T-test p-value^	Adjusted p-value*	Adjusted significance•	Percentage change$^{\Delta}$	Number of replicates	T-test p-value^	Adjusted p-value*	Adjusted significance•
Acetic acid 5%	-86	8	<0.001	0.003	Yes	-88	8	<0.001	<0.001	Yes
Acetic acid 2.5%	-90	8	<0.001	0.002	Yes	-91	8	<0.001	<0.001	Yes
Acetic acid 1.25%	-94	8	<0.001	0.002	Yes	-90	8	<0.001	<0.001	Yes
Acetic acid 0.63%	-94	8	<0.001	0.001	Yes	-94	8	<0.001	<0.001	Yes
Acetic acid 0.31%	-95	8	<0.001	0.001	Yes	-97	8	<0.001	<0.001	Yes
Acetic acid 0.16%	-96	8	<0.001	0.001	Yes	-86	8	0.001	0.011	Yes
Acetic acid 0.08%	-64	8	0.006	0.074	-	-23	8	0.035	0.491	-
Acetic acid 0.04%	+35	8	0.783	1.000	-	+5	8	0.653	1.000	-
Acetic acid 0.02%	+42	8	0.673	1.000	-	-7	8	0.298	1.000	-
Acetic acid 0.01%	+67	6	0.103	0.928	-	+11	4	0.589	1.000	-

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Table IV: Table showing the percentage (%) change in biofilm biomass for each of the *Acinetobacter baumannii* isolates when coincubated with each of the AMD, nAMD or AA for 72 hours, when compared to an untreated, positive control.

Dressing/agent	*A. baumannii* AYE					*A. baumannii* ACI_721				
	Percentage change[Δ]	Number of replicates	T-test p-value^	Adjusted p-value*	Adjusted significance•	Percentage change[Δ]	Number of replicates	T-test p-value^	Adjusted p-value*	Adjusted significance•
Mepilex® Ag	-95.9	8	<0.001	0.001	Yes	-100	6	<0.001	<0.001	Yes
Aquacel® Ag	-39	8	0.150	1.000	-	-74	6	<0.001	0.006	Yes
Aquacel® Ag Foam	-66	8	0.027	0.295	-	-74	6	0.001	0.010	Yes
Aquacel® Ag Burn	-94	6	0.002	0.031	Yes	-82	4	0.003	0.032	Yes
UrgoTul® Silver	-20	10	0.721	1.000	-	-4	8	0.970	1.000	-
Acticoat	-96	8	<0.001	0.002	Yes	-100	6	<0.001	<0.001	Yes
PolyMem Silver®	-61	8	0.007	0.090	-	-75	6	<0.001	0.001	Yes
Inadine®	+3	8	0.880	1.000	-	-6	6	0.820	1.000	-
L-Mesitran® Net	-10.4	6	0.469	1.000	-	+33	4	0.055	0.385	-
L-Mesitran® Hydro	-1	8	0.926	1.000	-	-62	6	0.004	0.038	Yes
Bactigras	-59	8	0.012	0.148	-	-68	6	0.004	0.038	Yes
UrgoTul® plain	-7	6	0.471	1.000	-	-27	4	0.068	0.405	-
PolyMem® plain	-66	6	0.004	0.054	-	-74	4	<0.001	0.003	Yes

Table legend: [Δ] where – refers to reduction in biofilm biomass, and + to increase in biofilm biomass ^ original p-values from the Student's T-test, * p-values adjusted for multiple comparisons using Holm's method, • column shows dressings with an adjusted p-value<0.05

Dressing/agent	*A. baumannii* AYE					*A. baumannii* ACI_721				
	Percentage change[Δ]	Number of replicates	T-test p-value^	Adjusted p-value*	Adjusted significance•	Percentage change[Δ]	Number of replicates	T-test p-value^	Adjusted p-value*	Adjusted significance•
Acetic acid 5%	-92	10	<0.001	<0.001	Yes	-90	8	<0.001	<0.001	Yes
Acetic acid 2.5%	-93	10	<0.001	0.001	Yes	-92	8	<0.001	<0.001	Yes
Acetic acid 1.25%	-93	10	<0.001	<0.001	Yes	-93	8	<0.001	<0.001	Yes
Acetic acid 0.63%	-93	10	<0.001	<0.001	Yes	-96	8	<0.001	<0.001	Yes
Acetic acid 0.31%	-93	10	<0.001	<0.001	Yes	-96	8	<0.001	<0.001	Yes
Acetic acid 0.16%	-90	10	<0.001	0.001	Yes	-94	8	<0.001	<0.001	Yes
Acetic acid 0.08%	-28	10	0.273	1.000	-	-95	8	<0.001	<0.001	Yes
Acetic acid 0.04%	+10	10	0.260	1.000	-	+5	8	0.404	1.000	-
Acetic acid 0.02%	+6	10	0.220	1.000	-	+13	8	0.157	0.787	-
Acetic acid 0.01%	-30	4	0.838	1.000	-	+7	4	0.541	1.000	-

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Figure legends

Figure 1: Graph showing the mean average biomass of the biofilms produced by the *A. baumannii* isolates as measured through the crystal violet assay

Optical density on the y axis refers to the average biofilm biomass for the *A. baumannii* isolates when tested with the range of agents shown on the x axis. All the data has been normalised by subtraction of the negative control and error bars (showing the standard error) have been provided. Test agents have been grouped according to the active antimicrobial agent present.

Figure 2: Graph showing the mean average biomass of the biofilms produced by the *P. aeruginosa* isolates as measured through the crystal violet assay

Optical density on the y axis refers to the average biofilm biomass for the *P. aeruginosa* isolates when tested with the range of agents shown on the x axis. All the data has been normalised by subtraction of the negative control and error bars (showing the standard error) have been provided. Test agents have been grouped according to the active antimicrobial agent present.

Figure 3: Graph showing the mean average biomass of the biofilms produced by the *A. baumannii* isolates as measured through the crystal violet assay

Optical density on the y axis refers to the average biofilm biomass for the *A. baumannii* isolates when tested with the AA and the best/worst performing dressings shown on the x axis. All the data has been normalised by subtraction of the negative control and error bars have been provided.

Figure 4: Graph showing the mean average biomass of the biofilms produced by the *P. aeruginosa* isolates as measured through the crystal violet assay

Optical density on the y axis refers to the average biofilm biomass for the *P. aeruginosa* isolates when tested with the AA and the best/worst performing dressings shown on the x axis. All the data has been normalised by subtraction of the negative control and error bars have been provided.