

## Antimicrobial dressings

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

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32 **ABSTRACT**

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

36 **Objectives**

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

40 **Methods**

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

44 **Results**

45 There is a large variation in ability of different dressings to inhibit biofilm formation, seen ~~both~~  
46 withbetween dressings that contain the same, and those that contain ~~a variety of~~ other  
47 antimicrobial agents. The best performing AMD were Mepilex<sup>®</sup> Ag and Acticoat. AA consistently  
48 prevented biofilm formation.

49 **Conclusions**

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

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

54 management.

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

## 56 1 INTRODUCTION<sup>1</sup>

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

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

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

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

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<sup>1</sup> 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.

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

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

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

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

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105 Silver (Ag) has been used extensively in burn wound management [15] and is a potent antimicrobial.  
106 Silver-containing dressings vary in their composition and act by a combination of i) absorbing wound  
107 exudates and killing the microorganisms drawn into the dressings, and/or ii) releasing active silver  
108 onto the wound bed. These biologically active ions then bind to negatively charged proteins, RNA,  
109 and DNA and damage bacterial cell walls, inhibit replication and reduce metabolism and growth [16].  
110 Broad antimicrobial activity has been reported against Gram-positive and Gram-negative organisms  
111 [17], protozoa, viruses [18], and fungi [19].

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

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



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

135 In addition to silver, AMD may contain iodine/povidone-iodine (which rapidly penetrates  
136 microorganisms, damaging proteins, nucleotides and fatty acids, leading to rapid cell death) [28],  
137 honey (which is antimicrobial due to osmotic effect, a low pH and the production of hydrogen  
138 peroxide [29]), or chlorhexidine; which binds to and disrupts the negatively charged bacterial cell  
139 wall and affects the osmotic equilibrium of the cell [30]. Furthermore, in addition to commercial  
140 AMD, ~~biocides~~other biocide-impregnated dressings may have a role to play in preventing wound  
141 infection. A range of biocides have been investigated in this regard (e.g. silver nitrate, mafenide  
142 acetate, povidine iodine, silver sulfadiazine and chlorhexidine), including acetic acid (CH<sub>3</sub>COOH).

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

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

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

155

## 156 | **2 METHODS**

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

163 | Four organisms were tested (two *Pseudomonas aeruginosa* and two *Acinetobacter baumannii*)  
164 | (Table I), and comprised well-characterised control strains (PS\_PA01, ACI\_AYE) and clinical isolates  
165 | from burns patients (ACI\_721, PS\_1586). All AM products (Table II) were freshly opened and were  
166 | within date when used. Experiments were performed using at least two biological replicates, and at  
167 | least four technical replicates of each isolate.

168

### 169 | **2.1 Processing of the AMDs**

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

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

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

178

## 179 **2.2 Impact of the AMD and AA on biofilm formation**

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

182 Overnight cultures of the test strains (grown in 5ml of Lysogeny (LB) broth [Oxoid]) were diluted in  
183 fresh antibiotic-free Muller-Hinton (MH) broth [Oxoid] to an optical density at 600nm (OD<sub>600</sub>) of 0.1,  
184 and then 1ml was seeded into wells of a 24-well MTT [Corning, New York], alongside 1ml of either  
185 diluted AA (water as diluent) or sterile water. AA was tested at the following dilutions: 5%, 2.5%,  
186 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  
187 dressing was placed into the well containing the organism suspension and water to provide a total  
188 volume of 2mls plus dressing.

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

191 Plates were sealed and statically incubated at (33°C); the temperature of the surface of a wound  
192 [35]. After 72 hours, the liquid and AMD pieces were removed from the wells and the plates rinsed  
193 in tap water to remove any unbound cells. Any existing biofilms were then visualised through  
194 staining with 2mls of 1% crystal violet (CV) [Sigma Aldrich, Poole, UK], further rinsed (as above) to  
195 remove unbound CV, and dye solubilised by the addition of 2mls of 70% ethanol. 200ul from each  
196 well was then transferred into wells of a 96-well microtitre tray, and the OD<sub>600</sub> of the solubilised CV

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

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

205

### 206 3 RESULTS

207 All four of the bacterial isolates (PS\_PA01, PS\_1586, ACI\_AYE and ACI\_721) were tested against all  
208 the AMD, nAMD and AA achieving at least four, but up to ten technical replicates. The  
209 ~~number~~numbers of replicates ~~can be seen in parenthesis in table~~are shown on tables III- and IV for *P.*  
210 *aeruginosa*, and *A. baumannii*, respectively.

211 The mean average optical densities of the solubilised CV were plotted per species for *A. baumannii*,  
212 and *P. aeruginosa*, and are shown in figures 1 and 2, respectively for the dressings, and figures 3 and  
213 4 for the AA and best/worst performing dressings against each species, respectively. The standard  
214 error bars (denoting variation in the number of technical replicates) are also plotted and all data has  
215 been normalised by subtraction of the negative (broth only) control.

216 The graphs demonstrate that there is a large variation in the test agents (AMD, nAMD and AA) in  
217 terms of reducing biofilm formation (e.g. from an increase of 33% with L-Mesitran<sup>®</sup> Net to a decrease  
218 of 100% with Acticoat and Mepilex<sup>®</sup> Ag for ACI\_721). This is seen ~~both with~~between different  
219 dressings that contain the same active agent (e.g silver) (e.g. from an increase of 43% with PolyMem  
220 Silver<sup>®</sup> to a decrease of 100% with Acticoat and Mepilex<sup>®</sup> Ag for PS\_PA01) and between those that

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

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

### 226 3.1 Performance of the silver-containing AMD

227 Seven silver-containing AMDs were tested and the majority showed some effectiveness at reducing  
228 biofilm formation (~~table~~tables III & IV, figures 1 & 2). For both species and all four isolates, Mepilex<sup>®</sup>  
229 Ag (Mölnlycke Healthcare), and Acticoat (Smith & Nephew) were highly effective, leading to 90-  
230 100% reduction of biofilm formation compared to the positive control. These results were highly  
231 consistent across all replicates as shown by the small error bars, and were also statistically significant  
232 in the t-tests with all p-values  $\leq 0.05$ .

233 For the Aquacel<sup>®</sup> dressings (Ag, Ag foam and Ag burn), the reductions were generally modest,  
234 averaging 44% for PS\_PA01 and 34% for PS\_1586. The *A. baumannii* isolates appear to be more  
235 susceptible to these dressings with average reductions of 77% for ACI\_721, and 65% for ACI\_AYE.

236 The results for Aquacel<sup>®</sup> Ag burn against biofilms of ACI\_AYE show reductions of 94% (statistically  
237 significant with adjusted p-values  $< 0.05$ ), and small standard error across all six replicates.

238 In general UrgoTul<sup>®</sup> Silver (a thin mesh-like AMD) was able to reduce biofilm formation for the  
239 majority of the isolates and replicates. However, for PS\_1586, for four of the eight replicates, there  
240 was an increase in biofilm biomass in wells containing the dressings. This ranged from 13-80% (data  
241 not shown). PolyMem Silver<sup>®</sup> also gave varied results, with reductions in biomass of biofilms  
242 apparent with the *A. baumannii* isolates (61% reduction for ACI\_AYE and 75% for ACI\_721), but  
243 increases noted with both the *P. aeruginosa* isolates (43% increase in biofilm biomass for PS\_PA01,  
244 and 11% increase for PS\_1586).

245

### 246 3.2 Performance of the non-silver containing AMD

247 The four non-silver containing AMD gave varied results. Inadine® (Systagenix), which contains  
248 povidone-iodine as the active agent, slightly reduced biofilm formation for the two clinical isolates  
249 (ACI\_721 and PS\_1586 and ACI\_721) by 6 and 10% respectively, but this was not statistically  
250 significant, and the dressing was ineffective against the control strains.

251 The honey-containing dressings of L-Mesitran® Net and L-Mesitran® Hydro (both from L-Mesitran  
252 Wound Care) were generally ineffective at preventing biofilm formation. Although reduced biofilm  
253 formation occurred with *A. baumannii* ACI\_AYE for both dressings with a maximum reduction of  
254 10.4% (not statistically significant, p values >0.05), and ACI\_721 (where there was a statistically  
255 significant reduction (adjusted p = 0.004038) of 62% in biofilm biomass with L-Mesitran® Hydro  
256 compared to the positive control), both dressings were ineffective at preventing biofilm formation of  
257 *P. aeruginosa*. For both isolates increased biofilm formation occurred, ranging from 20% with L-  
258 Mesitran® Net and PA\_1586 to 200% with L-Mesitran® Hydro and this same isolate. L-Mesitran®  
259 Hydro was the worst performer, with an average 115% increase in biofilm biomass for PS\_PA01, and  
260 average 200% increase for PS\_1586 (Table III).

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261 Bactigras (the only chlorhexidine-containing dressing) generally reduced biofilm formation, with  
262 ~~statistically significant~~ reductions of 39, 59 and 68% for PS\_PA01, ACI\_AYE and ACI\_721,  
263 respectively- (with this latter reduction statistically significant with an adjusted p-value of 0.038). It  
264 was however ineffective for PS\_1586, where there was an average 200% increase in biofilm biomass.

265

### 266 3.3 Performance of the AMD vs the nAMD

267 Despite not containing an AM agent, both of the nAMD reduced biofilm formation in this experiment  
268 for the *P. aeruginosa* (PS\_1586), and *A. baumannii* (ACI\_AYE and ACI\_721) isolates. Reductions

269 ranged from 7% to 74%, but were generally only statistically significant for ACI\_721, where the 74%  
270 reduction is associated with an adjusted p-value of 0.003 (table III) and IV. Some of the reductions  
271 were higher than those seen with some of the marketed AMDs. For example for ACI\_721,  
272 Inadine<sup>®</sup>, L-Mesitran<sup>®</sup> Net and L-Mesitran<sup>®</sup> Hydro resulted in differences of +3, -10.4, -6, +33, and -  
273 46.2%, compared to the nAMD (PolyMem<sup>®</sup> plain) where there was a 66.74% reduction in biofilm  
274 biomass (statistically significant, p=0.003).

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275 For PS\_PA01, there was no reduction in biofilm biomass with the nAMD (table III).

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### 277 3.4 Performance of the AMD compared to AA

278 The shaded cells in table tables III and IV highlight the data where there was reduction in biofilm  
279 formation by at least 90%. Acetic acid performs well as an anti-biofilming agent, with reductions of  
280 ≥90% seen for concentrations of AA from 5% down to 0.16% (0.08% for ACI\_721). This result was  
281 consistent across all replicates.

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

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

## 288 4 DISCUSSION

289 Medicated AMDs have the potential to significantly reduce bacterial contamination of burns and  
290 wounds; a post-insult complication that may delay wound healing, and lead to widespread systemic  
291 infection [3637]. Despite being a small study, this work has demonstrated that in the *in vitro* setting

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

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

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

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

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



316 that are released. In this study dressings were measured using a ruler and cut to 1cm<sup>2</sup>, but their  
317 volumes were not considered. A number of the dressings are thick foam (Mepilex<sup>®</sup> Ag, Aquacel<sup>®</sup> Ag  
318 Foam, PolyMem Silver<sup>®</sup> and PolyMem<sup>®</sup>), 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<sup>®</sup> Ag and Acticoat as 0.698 and 0.144 mg/cm<sup>2</sup>, respectively,  
324 compared to 0.00014 mg/cm<sup>2</sup> for PolyMem Silver<sup>®</sup>. 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.

342

343 **5 CONCLUSIONS**

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

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

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

358

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

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374

375 **7 Transparency declarations**

376 None to declare.

377

378 **8 Disclaimer**

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

Study Identifier	Organism	Description
PS_PA01	<i>Pseudomonas aeruginosa</i>	Control strain [ATCC_15692]. Originally isolated from an infected wound.
PS_1586	<i>Pseudomonas aeruginosa</i>	QEHB Clinical burn wound isolate.
ACI_AYE	<i>Acinetobacter baumannii</i>	Control strain [ATCC® BAA-1710]. Originally isolated from human blood.
ACI_721	<i>Acinetobacter baumannii</i>	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 <sup>®</sup> Ag [Silver]	Mölnlycke Healthcare	Silver sulphate (Ag <sub>2</sub> SO <sub>4</sub> ) 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 [4041]
Aquacel <sup>®</sup> Ag [Silver]	Convatec	Ionic silver impregnated hydrofibre pad composed of sodium carboxymethylcellulose and 1.2% ionic silver	AQUACEL <sup>®</sup> 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 <sup>®</sup> Ag Foam [Silver]	Convatec	As above	As above
Aquacel <sup>®</sup> Ag Burn [Silver]	Convatec	As above	As above
UrgoTul <sup>®</sup> Silver [Silver]	Urgo Medical	Hydrocolloid dressing consisting of a polyester web, impregnated with carboxymethyl 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 <i>in vitro</i> 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) [4243].
PolyMem Silver <sup>®</sup> [Silver]	Ferris MFG. Corp.	Polyurethane membrane matrix containing F68 surfactant, glycerol, a starch copolymer and silver.	
Inadine <sup>®</sup> [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 [4344].

Dressing [Agent]	Supplier	Antimicrobial agent and formulation	Reports/References
L-Mesitran <sup>®</sup> Net [Honey]	L-Mesitran Wound Care	Non-adherent open polyester mesh coated with a thin layer of L-Mesitran <sup>®</sup> Hydro gel.	L-Mesitran is a broad- <del>spectrumantimicrobialspectrum</del> antimicrobial, effective against most bacteria including MRSA and VRE [445]
L-Mesitran <sup>®</sup> 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, <i>Strep. pneumoniae</i> , <i>S. aureus</i> , <i>Proteus vulgaris</i> , <i>E. coli</i> , and <i>P. aeruginosa</i> [4546]
UrgoTul <sup>®</sup> plain [No AM agent]	Urgo Medical	n/a	n/a
PolyMem <sup>®</sup> plain [No AM agent]	Ferris MFG. Corp.	n/a	n/a
Acetic acid (5% stock)	Tayside Pharmaceuticals	Acetic acid ( CH <sub>3</sub> COOH)	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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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 number of replicates shown in parenthesis. Asterisks (\*) denote statistically significant changes in biomass (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 *Pseudomonas* nAMD or AA for 72 hours, when compared to an untreated, positive control.

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<u>Dressing/agent</u>	<u><i>P. aeruginosa</i> PA01</u>					<u><i>P. aeruginosa</i> PA 1586</u>				
	<u>Percentage change<sup>Δ</sup></u>	<u>Number of replicates</u>	<u>T-test p-value<sup>^</sup></u>	<u>Adjusted p-value<sup>*</sup></u>	<u>Adjusted significance<sup>•</sup></u>	<u>Percentage change<sup>Δ</sup></u>	<u>Number of replicates</u>	<u>T-test p-value<sup>^</sup></u>	<u>Adjusted p-value<sup>*</sup></u>	<u>Adjusted significance<sup>•</sup></u>
<u>Acetic acid 5%</u>	<u>-86</u>	<u>8</u>	<u>&lt;0.001</u>	<u>0.003</u>	<u>Yes</u>	<u>-88</u>	<u>8</u>	<u>&lt;0.001</u>	<u>&lt;0.001</u>	<u>Yes</u>
<u>Acetic acid 2.5%</u>	<u>-90</u>	<u>8</u>	<u>&lt;0.001</u>	<u>0.002</u>	<u>Yes</u>	<u>-91</u>	<u>8</u>	<u>&lt;0.001</u>	<u>&lt;0.001</u>	<u>Yes</u>
<u>Acetic acid 1.25%</u>	<u>-94</u>	<u>8</u>	<u>&lt;0.001</u>	<u>0.002</u>	<u>Yes</u>	<u>-90</u>	<u>8</u>	<u>&lt;0.001</u>	<u>&lt;0.001</u>	<u>Yes</u>
<u>Acetic acid 0.63%</u>	<u>-94</u>	<u>8</u>	<u>&lt;0.001</u>	<u>0.001</u>	<u>Yes</u>	<u>-94</u>	<u>8</u>	<u>&lt;0.001</u>	<u>&lt;0.001</u>	<u>Yes</u>
<u>Acetic acid 0.31%</u>	<u>-95</u>	<u>8</u>	<u>&lt;0.001</u>	<u>0.001</u>	<u>Yes</u>	<u>-97</u>	<u>8</u>	<u>&lt;0.001</u>	<u>&lt;0.001</u>	<u>Yes</u>
<u>Acetic acid 0.16%</u>	<u>-96</u>	<u>8</u>	<u>&lt;0.001</u>	<u>0.001</u>	<u>Yes</u>	<u>-86</u>	<u>8</u>	<u>0.001</u>	<u>0.011</u>	<u>Yes</u>
<u>Acetic acid 0.08%</u>	<u>-64</u>	<u>8</u>	<u>0.006</u>	<u>0.074</u>	<u>-</u>	<u>-23</u>	<u>8</u>	<u>0.035</u>	<u>0.491</u>	<u>-</u>
<u>Acetic acid 0.04%</u>	<u>+35</u>	<u>8</u>	<u>0.783</u>	<u>1.000</u>	<u>-</u>	<u>+5</u>	<u>8</u>	<u>0.653</u>	<u>1.000</u>	<u>-</u>
<u>Acetic acid 0.02%</u>	<u>+42</u>	<u>8</u>	<u>0.673</u>	<u>1.000</u>	<u>-</u>	<u>-7</u>	<u>8</u>	<u>0.298</u>	<u>1.000</u>	<u>-</u>
<u>Acetic acid 0.01%</u>	<u>+67</u>	<u>6</u>	<u>0.103</u>	<u>0.928</u>	<u>-</u>	<u>+11</u>	<u>4</u>	<u>0.589</u>	<u>1.000</u>	<u>-</u>

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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	<i>A. baumannii</i> AYE					<i>A. baumannii</i> ACL 721				
	Percentage change <sup>Δ</sup>	Number of replicates	T-test p-value <sup>Δ</sup>	Adjusted p-value*	Adjusted significance•	Percentage change <sup>Δ</sup>	Number of replicates	T-test p-value <sup>Δ</sup>	Adjusted p-value*	Adjusted significance•
Mepilex <sup>®</sup> Ag	-95.9	8	<0.001	0.001	Yes	-100	6	<0.001	<0.001	Yes
Aquacel <sup>®</sup> Ag	-39	8	0.150	1.000	-	-74	6	<0.001	0.006	Yes
Aquacel <sup>®</sup> Ag Foam	-66	8	0.027	0.295	-	-74	6	0.001	0.010	Yes
Aquacel <sup>®</sup> Ag Burn	-94	6	0.002	0.031	Yes	-82	4	0.003	0.032	Yes
UrgoTul <sup>®</sup> 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 <sup>®</sup>	-61	8	0.007	0.090	-	-75	6	<0.001	0.001	Yes
Inadine <sup>®</sup>	+3	8	0.880	1.000	-	-6	6	0.820	1.000	-
L-Mesitran <sup>®</sup> Net	-10.4	6	0.469	1.000	-	+33	4	0.055	0.385	-
L-Mesitran <sup>®</sup> 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 <sup>®</sup> plain	-7	6	0.471	1.000	-	-27	4	0.068	0.405	-
PolyMem <sup>®</sup> plain	-66	6	0.004	0.054	-	-74	4	<0.001	0.003	Yes

Table legend: <sup>Δ</sup> where – refers to reduction in biofilm biomass, and + to increase in biofilm biomass. <sup>Δ</sup> 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

<u>Dressing/agent</u>	<u>A. baumannii AYE</u>					<u>A. baumannii ACL 721</u>				
	<u>Percentage change<sup>Δ</sup></u>	<u>Number of replicates</u>	<u>T-test p-value<sup>^</sup></u>	<u>Adjusted p-value<sup>*</sup></u>	<u>Adjusted significance<sup>●</sup></u>	<u>Percentage change<sup>Δ</sup></u>	<u>Number of replicates</u>	<u>T-test p-value<sup>^</sup></u>	<u>Adjusted p-value<sup>*</sup></u>	<u>Adjusted significance<sup>●</sup></u>
<u>Acetic acid 5%</u>	<u>-92</u>	<u>10</u>	<u>&lt;0.001</u>	<u>&lt;0.001</u>	<u>Yes</u>	<u>-90</u>	<u>8</u>	<u>&lt;0.001</u>	<u>&lt;0.001</u>	<u>Yes</u>
<u>Acetic acid 2.5%</u>	<u>-93</u>	<u>10</u>	<u>&lt;0.001</u>	<u>0.001</u>	<u>Yes</u>	<u>-92</u>	<u>8</u>	<u>&lt;0.001</u>	<u>&lt;0.001</u>	<u>Yes</u>
<u>Acetic acid 1.25%</u>	<u>-93</u>	<u>10</u>	<u>&lt;0.001</u>	<u>&lt;0.001</u>	<u>Yes</u>	<u>-93</u>	<u>8</u>	<u>&lt;0.001</u>	<u>&lt;0.001</u>	<u>Yes</u>
<u>Acetic acid 0.63%</u>	<u>-93</u>	<u>10</u>	<u>&lt;0.001</u>	<u>&lt;0.001</u>	<u>Yes</u>	<u>-96</u>	<u>8</u>	<u>&lt;0.001</u>	<u>&lt;0.001</u>	<u>Yes</u>
<u>Acetic acid 0.31%</u>	<u>-93</u>	<u>10</u>	<u>&lt;0.001</u>	<u>&lt;0.001</u>	<u>Yes</u>	<u>-96</u>	<u>8</u>	<u>&lt;0.001</u>	<u>&lt;0.001</u>	<u>Yes</u>
<u>Acetic acid 0.16%</u>	<u>-90</u>	<u>10</u>	<u>&lt;0.001</u>	<u>0.001</u>	<u>Yes</u>	<u>-94</u>	<u>8</u>	<u>&lt;0.001</u>	<u>&lt;0.001</u>	<u>Yes</u>
<u>Acetic acid 0.08%</u>	<u>-28</u>	<u>10</u>	<u>0.273</u>	<u>1.000</u>	<u>-</u>	<u>-95</u>	<u>8</u>	<u>&lt;0.001</u>	<u>&lt;0.001</u>	<u>Yes</u>
<u>Acetic acid 0.04%</u>	<u>+10</u>	<u>10</u>	<u>0.260</u>	<u>1.000</u>	<u>-</u>	<u>+5</u>	<u>8</u>	<u>0.404</u>	<u>1.000</u>	<u>-</u>
<u>Acetic acid 0.02%</u>	<u>+6</u>	<u>10</u>	<u>0.220</u>	<u>1.000</u>	<u>-</u>	<u>+13</u>	<u>8</u>	<u>0.157</u>	<u>0.787</u>	<u>-</u>
<u>Acetic acid 0.01%</u>	<u>-30</u>	<u>4</u>	<u>0.838</u>	<u>1.000</u>	<u>-</u>	<u>+7</u>	<u>4</u>	<u>0.541</u>	<u>1.000</u>	<u>-</u>

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510 **Figure legends**

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

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

517

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

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

524

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

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

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532

533 **Figure 4: Graph showing the mean average biomass of the biofilms produced by the *P. aeruginosa***

534 **isolates as measured through the crystal violet assay**

535 Optical density on the y axis refers to the average biofilm biomass for the *P. aeruginosa* isolates

536 when tested with the AA and the best/worst performing dressings shown on the x axis. All the data

537 has been normalised by subtraction of the negative control and error bars have been provided.

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