In vitro activity of an engineered honey, medical-grade honeys, and antimicrobial wound dressings against biofilm-producing clinical bacterial isolates

Objective: Honey is recognised to be a good topical wound care agent owing to a broad-spectrum of antimicrobial activity combined with healing properties. Surgihoney RO (SH1) is a product based on honey that is engineered to produce enhanced reactive oxygen species (ROS) and has been reported to be highly antimicrobial. The objective was to investigate the ability of the engineered honey and its comparators to prevent biofilm formation in vitro.

Method: We tested the ability of three medical-grade honeys SH1, Activon manuka honey (MH) and Medihoney manuka honey (Med), alongside five antimicrobial dressings (AMDs) to prevent the formation of biofilms by 16 isolates. Honeys were serially double diluted from 1:3 down to 1:6144 and the lowest dilution achieving a statistically significant reduction in biomass of at least 50%, compared with untreated controls, was recorded.

Results: Although all the honeys were antibacterial and were able to prevent the formation of biofilms, SH1 was the most potent, with efficacy at lower dilutions than the medical honeys for five isolates, and equivalent dilutions for a further six. Additionally, SH1 was superior in antibacterial potency to three commercially available AMDs that contain honey.

Conclusion: SH1 is effective at preventing biofilms from forming and is superior to medical honeys and AMDs in in vitro tests.

Declaration of interest: Surgihoney RO was provided free of charge for testing by Matoke Holdings, UK and the hospital pharmacy provided the other honeys and dressings. This paper presents independent research funded by the National Institute for Health Research (NIHR). The views expressed are those of the authors and not necessarily those of the NHS, the NIHR or the Department of Health.
that SH1 has microbicidal action against both Gram-positive and Gram-negative bacterial wound isolates, and have indicated that it is superior to other honeys in terms of potency. In one study, Dryden et al.,13 tested the in vitro efficacy of the formulations of SH against bacterial wound isolates by determining the minimum inhibitory concentrations (MICs), and minimum bactericidal concentrations (MBCs), and compared these results with those obtained from a variety of other honeys. A total of 48 isolates were tested (comprising Staphylococcus aureus (MRSA and MSSA), ß-haemolytic streptococci, Enterococcus spp, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, along with a range of other Gram-positive organisms and fungi) and the results demonstrated that all the SH formulations had higher antimicrobial activity than the other honeys tested, as evidenced by the larger inhibitory zones present around the test honey using an agar diffusion method. Time-kill experiments in this same study further demonstrated the speed of the cidal activity of SH, with undetectable bacterial growth (for a range of Gram-positive and Gram-negative organisms) within 30 minutes for the most potent prototype SH formulation, and within 2 hours for the currently marketed and licensed version.

Clinical outcomes have also been favourable with SH1. In a pilot study looking at the prevention of surgical site infection (SSI) in patients undergoing caesarean section (CS) surgery,12 SH1 was offered to all patients who had undergone a CS. Recipients were then monitored over 30 days for the development of infection, and rates of infection were compared with a previous cohort of patients who received no SH1. It reduced SSI by 60% compared with normal wound dressings, and was found to offer considerable cost savings over other preparations.12 Further favourable outcomes were observed when SH1 was used to reduce bacterial colonisation in long lines in oncology patients.14

Although these in vitro and clinical studies provide good evidence to support the antimicrobial activity of SH1, no work has yet been performed to assess the activity of this engineered honey against biofilms. Biofilms are associated with wound chronicity, and are present in 60% of those with chronic wound infections.15

This in vitro study was undertaken to assess whether SH1 has any antibacterial activity against biofilms, in terms of ability to prevent their formation. To evaluate this, SH1 was tested on a range of biofilm-producing bacteria and the activity compared against two standard medical-grade honeys and commercially available honey- and silver-containing antimicrobial wound dressings.

### Methods

In vitro experiments were conducted on a panel of important wound pathogens (Table 1) to determine the antibacterial effects of Surgihoney RO (Matoke Holdings, UK), MediHoney (Derma Sciences, UK) and Activon tube 100% medical grade Manuka honey (Advancis Medical, UK), these will be referred to as SH1, Med and MH, respectively.

There were five antimicrobial dressings (AMDs) tested alongside SH1 on a limited panel of the isolates, so that any observed effects of SH1 could be compared with commercially available dressings containing honey, or alternative antimicrobial agents. The selection of honey-containing AMDs reflects those available in the formulary at our hospital, a major tertiary referral centre.

A total of 16 organisms (previously identified as good biofilm-producers)16 were tested against the three honey formulations (Table 1):

- Four Pseudomonas aeruginosa
- Four Acinetobacter baumannii
- Four Staphylococcus aureus
- Three multidrug resistant organisms (MDR). Klebsiella pneumoniae (carbapenem-resistant Enterobacteriaceae), Escherichia coli (ESBL positive), Pseudomonas aeruginosa (carrying the Verona integron-encoded ß-lactamase (VIM) plasmid conferring resistance to the carbapenems)
- Standard Escherichia coli isolate for reference.

<table>
<thead>
<tr>
<th>Study Identifier</th>
<th>Organism Description</th>
<th>Organism</th>
</tr>
</thead>
<tbody>
<tr>
<td>PS_PA01</td>
<td>Pseudomonas aeruginosa</td>
<td>ATCC_15692</td>
</tr>
<tr>
<td>PS_6749</td>
<td>Pseudomonas aeruginosa</td>
<td>NCTC_6749</td>
</tr>
<tr>
<td>PS_1054</td>
<td>Pseudomonas aeruginosa</td>
<td>QEHB clinical burn isolate</td>
</tr>
<tr>
<td>PS_1586</td>
<td>Pseudomonas aeruginosa</td>
<td>QEHB clinical burn isolate</td>
</tr>
<tr>
<td>ACI_AYE</td>
<td>Acinetobacter baumannii</td>
<td>MPR clinical isolate (Paris)</td>
</tr>
<tr>
<td>ACI_1960</td>
<td>Acinetobacter baumannii</td>
<td>ATCC_1960</td>
</tr>
</tbody>
</table>
### Table 2. List of the dressings/agents used in this study, their supplier, antimicrobial agent and formulation, and reports on their activity

<table>
<thead>
<tr>
<th>Dressing/agent</th>
<th>Supplier</th>
<th>Antibacterial agent and formulation</th>
<th>Reports/references</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surgihoney RO (Honey and enhanced production of hydrogen peroxide)</td>
<td>Matoke Holdings, UK</td>
<td>Natural honey (not a manuka honey) that has been engineered to produce high levels of reactive oxygen species (ROS)</td>
<td>Research shows it is highly active against a range of Gram-positive and Gram-negative bacteria, including multidrug resistant ones. More potent than other honeys, including Medihoney.13</td>
</tr>
<tr>
<td>Activon tube 100% medical-grade manuka honey (Honey)</td>
<td>Advancis Medical</td>
<td>100% Manuka Honey (without additives) from New Zealand</td>
<td>Biofilms of Staphylococcus aureus, MRSA and VRE were prevented and inhibited in vitro at concentrations that could be used in clinical practice.28</td>
</tr>
<tr>
<td>Medihoney ‘antibacterial medical honey’ (Honey)</td>
<td>Derma Sciences</td>
<td>Medical-grade honey predominantly sourced from Leptospermum species (Manuka honey)</td>
<td>The minimum inhibitory concentration (MIC) was tested against 130 clinical strains and it was effective at concentrations ranging from 4–14% v/v. Organisms tested included Staphylococcus aureus, Pseudomonas aeruginosa, Enterococcus faecalis, Klebsiella pneumoniae, Enterobacter spp., and Acinetobacter baumannii. It was also effective at preventing the formation of Pseudomonas aeruginosa biofilms in vitro when used at concentrations attainable in clinical use.30</td>
</tr>
<tr>
<td>Aquacel Ag (Silver)</td>
<td>Convatec</td>
<td>Ionic silver impregnated hydrofibre pad composed of sodium carboxymethylcellulose and 1.2% ionic silver</td>
<td>Contain ionic silver to kill a wide variety of microorganisms (including certain tested antibiotic-resistant bacteria), within 30 minutes, and provide sustained bacterial killing for up to seven days.31,32</td>
</tr>
<tr>
<td>Aquacel Ag+ extra (Silver)</td>
<td>Convatec</td>
<td>Ionic silver impregnated hydrofibre pad composed of a combination of silver and ‘anti-biofilming agents’</td>
<td>The ionic silver in the dressing kills pathogenic microorganisms, both planktonic and within bacterial biofilms, including wound bacteria, yeasts and moulds. The dressing also disrupts and absorbs biofilm, prevents biofilm formation/reformation and increases the efficiency of silver transfer to microorganisms.31,32</td>
</tr>
<tr>
<td>Actilite (Honey)</td>
<td>Advancis Medical</td>
<td>Light viscose net dressing coated with antibacterial Manuka honey and Manuka oil</td>
<td>The antibacterial effect has been enhanced by combining high grade antibacterial Manuka oil with Manuka honey. Dressing has been demonstrated in vitro to be effective against MRSA, VRE and Providencia stuartii.33</td>
</tr>
<tr>
<td>L-Mesitran Net (Honey)</td>
<td>L-Mesitran Wound Care</td>
<td>Non-adherent open polyester mesh coated with a thin layer of L-Mesitran Hydro gel.</td>
<td>L-Mesitran is a broad-spectrum antimicrobial, effective against most bacteria including MRSA and VRE.</td>
</tr>
<tr>
<td>L-Mesitran Hydro (Honey)</td>
<td>L-Mesitran Wound Care</td>
<td>Hydrogel sheet (1mm thick) attached to a semi-polyurethane membrane by a thin fibrous bonding layer. The hydrogel contains 30% medical-grade honey.</td>
<td>As above.</td>
</tr>
</tbody>
</table>

The panel contained a mixture of well-characterised control strains (PS_PA01, PS_6749, ACI_C59, ACI_C60, ACI_19606, EC_042, MSSA_10788, MSSA_F77, MRSA_F475, MRSA_F483, and MDR_C) and clinical isolates from burns patients with large open wounds (PS_1054, PS_1586, ACI_AYE, MDR_B, MDR_D), and represent a diverse range of strains (data not shown).

The isolates were all varied in terms of antibiotic (data not shown), stored at −80°C on Protect beads, and were cultured on cysteine lactose electrolyte deficient (CLED) agar, or blood agar (as appropriate; Biomerieux, France) before each experiment. Honeys and AMDs (Table 2) were freshly opened and within date when used. Experiments were performed using at least two biological replicates, and at least six technical replicates of each isolate per test dilution.
Preparation of the honey

The honeys were prepared in the same way and tested at a range of dilutions from 1:3 down to 1:6144. The strongest concentration (1:3) was made by placing 6ml of honey into a universal tube and adding 14ml of water to make a total volume of 20ml, which was then serially diluted down in sterile autoclaved distilled water until 1:6144 was reached. Dilution from the neat product was necessary since the viscosity of the neat honey meant that it was impossible to standardise the volume used in the experiments at this concentration. The dilution chosen (1:3) was the most concentrated solution that could be accurately pipetted into the test wells.

Impact of the honey on biofilm formation

The ability of the honey formulations to prevent biofilm formation was assessed using a crystal violet (CV) biofilm formation assay with the endpoint measurement being the ‘minimum biofilm inhibitory concentration’ (MBIC).

Overnight cultures of the test strains (grown in 5ml of Lysogeny broth (LB; Oxoid)) were diluted to an optical density at 600nm (OD600) of 0.1, and then 100μl seeded into wells of a 96-well microtiter plate (Corning, New York), with 1ml of either diluted honey (with water as diluent) or sterile distilled water. The honeys were tested at the following dilutions: 1:3, 1:6, 1:12, 1:24, 1:48, 1:96, 1:192, 1:384, 1:768, 1:1536, 1:3072, and 1:6144. Suitable controls were included in each assay, comprising 100μl overnight bacterial culture with 100μl water (for the positive control) or 200μl Muller-Hinton broth alone (for the negative control). This composition of the positive control was selected so that both the positive control and the test wells contained the same volume and concentration of bacteria, and the same amount of broth. Two biological and three technical replicates were performed for each strain and each honey dilution, respectively.

Plates were sealed and statically incubated at 33°C, the temperature of the surface of a wound. After 72 hours, the liquid was 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 200μl of 1% CV (Sigma Aldrich, Poole, UK), further rinsed (as above) to remove unbound CV, and dye solubilised by the addition of 200μl of 70% ethanol. The OD600 of the solubilised CV solution was then measured using a FLUOstar Optima (BMG Labtech) to assess the biomass of the biofilms.

If positive and negative controls for each test plate were within a normal range, the rest of the data were analysed for statistical significance by comparing values at each dilution of honey with untreated (positive) controls using the students’ t-test. The MBIC was defined as the lowest dilution of honey where there was both statistical significance in the t-test (p value <0.05) and a prevention of biofilm biomass accumulation ≥50% compared with the positive control.

Preparation of antimicrobial dressings

The following AMD were prepared for testing: Aquacel Ag, Aquacel Ag+ extra (both Convatec), Actilite (Advancis Medical), L-Mesitran Net, and L-Mesitran Hydro (both from L-Mesitran Wound Care). These dressings were chosen as they either i) represent the most commonly used silver dressings in our burns unit (Aquacel Ag, and Aquacel Ag + extra), or ii) are composed of honey (Actilite, L-Mesitran Net, and L-Mesitran Hydro).

Each sterile dressing was carefully cut into a number of 1cm² pieces (sufficient for one piece per test well) using a sterile scalpel or a pair of flame-sterilised scissors. Details of these dressings (and references to published work) are in Table 2.

Impact of antimicrobial dressings on biofilm formation

 Overnight cultures of the test strains (grown in 5ml of Lysogeny broth (LB; Oxoid)) were diluted to an optical density at 600nm (OD600) of 0.1, and then 100μl seeded into wells of a 96-well microtiter tray (MTT) (Fisher Scientific), alongside 100μl of either diluted honey (with water as diluent) or sterile distilled water. The honeys were tested at the following dilutions: 1:3, 1:6, 1:12, 1:24, 1:48, 1:96, 1:192, 1:384, 1:768, 1:1536, 1:3072, and 1:6144. Suitable controls were included in each assay, comprising 100μl overnight bacterial culture with 100μl water (for the positive control), or 200μl Muller-Hinton broth alone (for the negative control). This composition of the positive control was selected so that both the positive control and the test wells contained the same volume and concentration of bacteria, and the same amount of broth. Two biological and three technical replicates were performed for each strain and each honey dilution, respectively.

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If positive and negative controls for each test plate were within a normal range, the rest of the data were analysed for statistical significance by comparing values at each dilution of honey with untreated (positive) controls using the students’ t-test. The MBIC was defined as the lowest dilution of honey where there was both statistical significance in the t-test (p value <0.05) and a prevention of biofilm biomass accumulation ≥50% compared with the positive control.

References

% change in biofilm biomass has been reported for the AMD data (Table 4).

**Impact of the honeys on biofilm formation**

SH1 was able to prevent biofilm formation for all 16 of the isolates, with MBIC values ranging from 1:6 (PS_PA01 and PS_1586) to 1:192 (MDR_C and MRSA_F483) (Table 3). The dilution of 1:12 was the most commonly observed MBIC value and was present in six of the isolates (two *Pseudomonas aeruginosa* and four *Acinetobacter baumannii*). The remaining Gram-negative isolates were highly susceptible to SH1, with MBICs of 1:24 (MDR_D), 1:48 (MDR_B), and 1:96 for EC_042. Similar MBICs were seen with the Gram-positive isolates; 1:24 (MSSA_10788), 1:48 (MRSA_F475, MSSA_F77), and 1:192 (MRSA_F483).

MH was able to prevent biofilm formation of 14 of the 16 isolates, but was ineffective for two isolates (PS_PA01 and MRSA_F475). Here, there was no statistically significant prevention of biomass accumulation even when the strongest dilution of honey was used (1:3). This finding was repeatable.

Of the 14 isolates, MH was effective at preventing biofilms for eight (PS_1054, PS_6749, ACI_AYE, MDR_C, EC_042, MSSA_10788, MRSA_F483, and MSSA_F77) when used at the same dilution as SH1 (denoted by * Table 3), but required a stronger dilution than SH1 for the remaining six isolates (PS_1586, MRSA and VRE involves hydroxyl radicals generated from honey's hydrogen peroxide. Front Microbiol 2012; 3: 36.


16 Halstead, F.D., Rauf, M., Bamford, A. et al. Antimicrobial dressings: Comparison of the ability of a panel of dressings to prevent biofilm formation by key burn wound pathogens. Burns 2015; in press. 41: 8, 1683–1694.


20 Filan, J., Bohova, J., Hornackova, M. et al. Anti-biofilm effects of honey against wound pathogens *Proteus mirabilis* and *Enterobacter cloacae*.


25 White, J.W., Subers, M.H., Scheperz, A.I. The identification of inhibine, the antibacterial factor in honey, as hydrogen peroxide and its origin in a honey glucose-oxidase system. Biochem Biophys Acta 1963; 73, 57–70.


28 Cooper, R., Jenkins, L., Rowlands, R. Inhibition of biofilms through the use of manuka honey. Wounds UK 2011; 7: 1, 24–32.

29 George, N.M., Cutting, K.F. Antibacterial Honey (Medihoney®); in-vitro Activity Against Clinical Isolates of MRSA, VRE, and Other Multiresistant Gram-negative Organisms Including Pseudomonas aeruginosa. Wounds 2007; 19: 9, 231–236.

30 Cooper, R., Jenkins, L., Hooper, S. Inhibition of biofilms of pseudomonas aeruginosa by Medihoney in vitro. Wound Care 2014; 23: 3, 93–104.

31 Physical disruption of biofilm by Aquacel Ag wound dressing. Scientific Background Report.

Med was ineffective against biofilm production by one isolate (PS_PA01), where there was no statistically significant reduction in biofilm biomass, even when the strongest dilution of honey was used (1:3).

With Med, five of the isolates had an MBIC of ≤1:6 (all Acinetobacter baumannii isolates, and MDR_D), which is similar to MH where five of the isolates also have MBICS of ≤1:6. The highest MBIC observed with Med was with PS_6749, where the lowest effective concentration to inhibit biofilm formation was 1:1536. This was statistically significant (p=0.009) and there was a greater than 50% reduction in biofilm biomass compared with the positive control (data not shown).
Comparison of the honeys

Although all the honeys were antibacterial and able to prevent the formation of biofilms for the majority of the isolates tested, SH1 was generally the most potent. SH1 was effective at lower dilutions than both the other honeys for five of the isolates (ACI_C59, ACI_C60, ACI_19606, MDR_B, and MDR_D), and was equivalently effective to the other honeys for a further six isolates (PS_1054, MDR_C, EC_042, MSSA_10788, MRSA_F483, and MSSA_F77). For the remaining five isolates, SH1 was either the only effective honey (PS_PA01), was one of two effective honeys (MRSA_F475), or gave concordant/discordant results compared with MH and Med (PS_1586, PS_6749).

Generally, MH and Med have similar MBIC for all isolates. However, for ACI_AYE, MH was superior to Med, and could prevent biofilm formation at 1:12 (the same effective dilution as SH1), compared with 1:3 for Med. There was also a difference in MBIC between MH (MBIC of 1:6) and Med (MBIC of 1:3) for MDR_B. All these results are statistically significant (p<0.05).

On two occasions (PS_1586 and PS_6749), Med demonstrated superior potency to the other honeys. For the latter, the MBIC was 1:1536 (p=0.009), compared with 1:12 for both SH1 and MH (Fig 4). This may be explained by the enhanced growth at 1:24 observed with SH1 and MH for this isolate.

Comparison of the antibacterial activity of engineered honey and the antimicrobial dressings

The activity of SH1 was additionally tested and compared with a range of AMDs. This honey was chosen owing to the higher potency observed early on in the experiments, as it was not logistically possible to test all honeys in this experiment.

Pseudomonas aeruginosa isolates, PS_PA01, PS_1054, PS_1586, and PS_6749 were tested against SH1 and the range of AMD, achieving at least two, and up to six replicates per isolate (Table 4). The % change in biofilm biomass with each dressing or SH1 dilution was calculated based on the untreated positive control and are listed alongside those that were statistically significant (p<0.05; Table 4). Fig 3 shows this information with the statistically significant reduced accumulations of biomass denoted by an asterisk.

These data show that there is a large variation in the ability of the test agents to prevent the formation of biofilms of the Pseudomonas isolates tested. SH1 was effective at preventing biofilm formation of all isolates when used at a dilution of 1:3 with reductions in accumulations ranging from 79% (with PS_1586) to 94.1% (with PS_PA01). All of these reductions are statistically significant (p<0.05).

In fact, SH1 still resulted in reduced biofilm formation that was statistically significant when used down to 1:6 (where there was an 82% reduction for
Table 4. Table showing the % change in biofilm biomass for each of the Pseudomonas aeruginosa isolates when incubated with SH1 and each of the antimicrobial dressings for 72 hours, compared with an untreated, positive control

<table>
<thead>
<tr>
<th>Pseudomonas aeruginosa PS_PA01</th>
<th>Pseudomonas aeruginosa PS_1054</th>
<th>Pseudomonas aeruginosa PS_1586</th>
<th>Pseudomonas aeruginosa PS_6749</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% change</td>
<td>n</td>
<td>p</td>
</tr>
<tr>
<td>Aquacel Ag</td>
<td>−38.4</td>
<td>3</td>
<td>0.33</td>
</tr>
<tr>
<td>Aquacel Ag + Extra</td>
<td>−95.3</td>
<td>4</td>
<td>0.007</td>
</tr>
<tr>
<td>Actilite</td>
<td>+62.3</td>
<td>4</td>
<td>0.063</td>
</tr>
<tr>
<td>L-Mesitran Net</td>
<td>−27.8</td>
<td>4</td>
<td>0.132</td>
</tr>
<tr>
<td>L-Mesitran Hydro</td>
<td>−15.8</td>
<td>4</td>
<td>0.488</td>
</tr>
<tr>
<td>SH1:3</td>
<td>−94.1</td>
<td>6</td>
<td>0.005</td>
</tr>
<tr>
<td>SH1:6</td>
<td>−82.8</td>
<td>6</td>
<td>0.01</td>
</tr>
<tr>
<td>SH1:12</td>
<td>−34.9</td>
<td>6</td>
<td>0.16</td>
</tr>
<tr>
<td>SH1:24</td>
<td>+22</td>
<td>6</td>
<td>0.25</td>
</tr>
<tr>
<td>SH1:48</td>
<td>+4.5</td>
<td>6</td>
<td>0.81</td>
</tr>
<tr>
<td>SH1:96</td>
<td>−1.6</td>
<td>6</td>
<td>0.93</td>
</tr>
<tr>
<td>SH1:1:92</td>
<td>−32.1</td>
<td>6</td>
<td>0.68</td>
</tr>
<tr>
<td>SH1:3:84</td>
<td>−34.2</td>
<td>6</td>
<td>0.184</td>
</tr>
<tr>
<td>SH1:7:68</td>
<td>−19.6</td>
<td>6</td>
<td>0.39</td>
</tr>
<tr>
<td>SH1:1:1536</td>
<td>−11.4</td>
<td>6</td>
<td>0.58</td>
</tr>
</tbody>
</table>

% Change of biofilm biomass compared with an untreated positive control; SHI–Surgihoney RO; ‡p values from Student's T-test

PS_PA01 and 1:12 (with a 57% reduction for PS_1054). When SH1 was diluted beyond these values, there were no statistically significant reductions in biofilm formation (Fig 3).

Of the AMD, Aquacel Ag, and Aquacel Ag + extra (the silver-containing dressings) were most effective at preventing biofilm formation for the majority of isolates. Apart from the result for PS_PA01, Aquacel Ag was associated with reductions of 84–95%, and the reduction was statistically significant for PS_6749, and PS_1054. Similar results were obtained with Aquacel Ag + extra. The performance of the honey-containing dressings (Actilite, L-Mesitran Net, and L-Mesitran Hydro) was disappointing, with average increases in biofilm formation of 98% for all isolates with Actilite (range: 62–155.6%), and increases in biofilm biomass for three isolates (PS_1054, PS_1586 and PS_6749) with L-Mesitran Net, and L-Mesitran Hydro (Table 4).
Discussion

Although several studies have shown the antibacterial effects of a range of medical-grade honeys on biofilms, none have tested SH1: an engineered natural honey with enhanced production of ROS. Through a series of in vitro experiments on a panel of 16 clinically important burn-wound pathogens, we have shown that all the honeys (SH1, MH and Med) are able to reduce biofilm formation in vitro, but that the MBICs differ per honey and per organism.

Generally, the Gram-negative organisms were most susceptible to the honeys, including those MDR organisms. SH1 was the most potent of all the honeys, being effective at lower concentrations (weaker dilutions) than those required for MH and Med, and compared favourably against the two commonly used silver dressings. SH1 was also more effective at preventing biofilm formation in vitro of four Pseudomonas aeruginosa isolates than the three commercially available honey-containing dressings. While we did not conduct an extensive evaluation against all available honey-containing AMDs, we have compared all those available in our hospital that contain honey of some variety.

Honeys (including those of medical grade) are diverse, and it is known that potency can vary as much as 100-fold. Cooke et al. showed that the antibacterial activity of SH1 was due to the generation of H2O2 and reactive oxygen species (ROS), and that release was sustained over a period of at least 24 hours. H2O2 is produced on dilution of the honey by the action of glucose oxidase, and reaches maximum levels when the honey is diluted to between 50% and 30% w/v. Sustained ROS activity in SH1 has been demonstrated in vitro for over 3 days through the use of peroxide testing strips. Of note, MH is a non-peroxide honey and H2O2 has been shown to be absent. This may explain the differences in potency between the three honeys.

SH1 represents a highly effective and promising topical antibacterial as evidenced by both in vitro experiments, and in vivo.

A Cochrane review into ‘honey as a topical treatment for wounds’ concluded that there is high-quality evidence that honey accelerates the healing of partial-thickness burn wounds compared with conventional dressings, and is also more effective than antiseptic for treating infected surgical wounds. The evidence for the clinical benefits of honey for wound healing, however, was equivocal, although SH1 was not available for inclusion in the referenced studies. These data presented in this and other studies strongly support the use of SH1 as an antibacterial, and suggest further clinical evaluation is warranted. A rigorously controlled clinical trial is currently being planned at our centre to investigate the clinical use of SH1 further.

Limitations

However, there are limitations that must be considered. For example, the in vitro experiments all involved single species of bacteria, which have been artificially grown in the presence of the required nutrients. Biofilms in the clinical setting are likely to be vastly different in terms of their composition (for example they are likely to be composed of multiple bacterial species) and physiology (in terms of metabolic rate and presence of nutrients). Our data have shown that SH1 can prevent biofilm formation in vitro but not the impact it has on established biofilms. Preliminary work we have performed, has shown that SH1 can reduce the seeding (the release of planktonic bacterial cells from the biofilm surface so that new sites can be colonised) of pre-formed biofilms of the same panel of 16 isolates, after 24-hour exposures. Therefore, some evidence exists that honey can disrupt biofilms in vitro (Halstead et al. unpublished), however comparable in vivo activity against biofilms remains to be tested.

The in vitro data from this study also highlights that for some isolates (PS_1054, PS_1586, PS_6749, EC_042 and MSSA_10788) the test honey may actually enhance growth at certain dilutions. For these isolates, all honeys were equally likely to enhance biofilm growth with this typically occurring at values lower than the MBIC (1:24 for the Pseudomonas isolates, 1:96 for MSSA_10788, and 1:192 for EC_042). This may prove problematic in the clinical setting if the honey is applied at an effective concentration but is then diluted down to subinhibitory concentrations by wound exudates, or other secretions from the wound surface. An additional limitation is that the majority of clinical reports to date are anecdotal reports of patients with chronic wounds recovering after application of SH1—it would need to be demonstrated that this was due to the SH1 and not simply because the wound was already healing.

Conclusions

The work presented here supports previous in vitro findings and is consistent with the anecdotal clinical evidence. All the honeys tested, SH1, MH and Med, demonstrated antibacterial activity against the formation of biofilms. However, SH1, appears to be the most potent against a range of Gram-positive and Gram-negative bacteria, including MDR organisms.

Although further randomised controlled trials are required, SH1 clearly has considerable therapeutic and infection-prevention potential for the management and treatment of chronically colonised and infected wounds, and may help to reduce the use of antibiotics and selection pressure while promoting wound healing and infection prevention.