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Engineered honey: In vitro antimicrobial activity of a novel topical wound care treatment

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ABSTRACT

SurgihoneyRO™ is a novel engineered organic honey product for wound care. Its antimicrobial activity can be controlled and adjusted by the engineering process, allowing preparation of three different potencies, labelled SurgihoneyRO™ 1–3. Susceptibility testing of a range of wound and ulcer bacterial isolates to SurgihoneyRO™ by the disc diffusion method, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) determination, and time–kill measurements by time suspension tests were performed. SurgihoneyRO™ demonstrated highly potent inhibitory and cidal activity against a wide range of Gram-positive and Gram-negative bacteria and fungi. MICs/MBCs were significantly lower than concentrations likely to be achieved in topical clinical use. The topical concentration of SurgihoneyRO™ in wounds was estimated at ca. 500 g/L. MICs/MBCs for *Staphylococcus aureus* were 32/125 g/L for SurgihoneyRO™ 1 and 0.12/0.25 g/L for SurgihoneyRO™ 3. Cidal speed depended on potency, being 48 h for SurgihoneyRO™ 1 and 30 min for SurgihoneyRO™ 3. Maintenance of the SurgihoneyRO™ inoculum preparation for up to a week demonstrated complete cidal activity and no bacterial persistence. SurgihoneyRO™ has wide potential as a highly active topical treatment combining the effects of the healing properties of honey with the potent antimicrobial activity of the engineered product for skin lesions, wounds, ulcers and cavities. It is highly active against multidrug-resistant bacteria. It is more active than other honeys tested and is comparable with chemical antiseptics in antimicrobial activity.

1. Introduction

Honey has been used for millennia as topical wound therapy based on observations of its healing properties and cleansing action on suppurating wounds [1–4]. This use of honey may have been based on further observations that honey in hives does not deteriorate or become contaminated [5,6]. The clinical burden of soft tissue damage is increasing. Superficial wounds and skin ulcers are becoming increasingly common with the rising age of the population in many countries and the global epidemic of obesity and type 2 diabetes [7]. In the UK, community nurses spend much of their time dressing leg ulcers, and supervision by leg ulcer nurses is essential if standards are to be maintained in community leg ulcer services [8]. Most chronic breaks in the skin become colonised with bacteria [9–11]. It is difficult to know when and whether these bacteria are pathogenic, but it is likely that even if overt infection is not present, bacterial colonisation plays a role in slowing tissue healing, allowing the establishment of biofilm and resulting in wound slough and an offensive odour [12,13].

Tissue viability is challenging, particularly when complicated by co-morbidities [14]. Chronic wounds always become colonised with bacteria, which may destabilise the healing process [9–13]. There is a temptation to send a microbiological sample and to offer systemic antibiotics when the sample is reported as growing bacteria. All this serves is to select ever more resistant microbes, which is why chronic lower extremity ulcers are so often colonised with multidrug-resistant (MDR) organisms such as meticillin- resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aerugi- nosa* [15].

SurgihoneyRO™ is a licensed sterile product that has been developed as a dressing for wounds. It consists of natural honey sourced from several sites that has been through a process to produce different potencies of antimicrobial activity which greatly exceed the activity of other honey dressings. It is comparable with chemical antisepsis but appears to retain the wound healing properties of natural honey [1–3].

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This study examined the in vitro properties of this novel engineered product based on natural honey that has been through a process which enhances its antimicrobial properties. Different grades of the product (SurgihoneyRO™ 1–3) can be produced with increasing levels of antimicrobial potency. This level of antimicrobial activity can be replicated and is stable. Each of these SurgihoneyRO™ grades may have a role in topical clinical treatment depending on the degree of antimicrobial activity required. This is an entirely novel process and product. As an engineered product, SurgihoneyRO™ retains all of the established healing properties of natural honey, but its antimicrobial activity can be set at whichever potency is required. SurgihoneyRO™ 1 is a sterilised, pharmaceutical grade product licensed for clinical use as a topical wound dressing in the UK. The other grades, 2 and 3, are currently prototype products. This study aimed to establish the in vitro efficacy of the SurgihoneyRO™ grades against bacterial wound isolates by determining minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) of SurgihoneyRO™ 1, 2 as well as 3 and time–kill curves by suspension tests.

2. Materials and methods

2.1. SurgihoneyRO™

SurgihoneyRO™ was provided by the manufacturer (Healing Honey Ltd., Bicester, UK) as potency grades 1, 2 and 3. The product grades were presented as a sterile product in a sachet in semisolid form.

2.2. Clinical isolates

Clinical isolates were collected from soft tissue microbiology samples submitted to the Microbiology Department of Hampshire Hospitals Foundation Trust (Winchester, UK). Eighteen isolates of *S. aureus* (12 meticillin-sensitive *S. aureus* and 6 MRSA), six isolates of b-haemolytic streptococci [Lancefield groups A (2), B (2), C (1) and G (1)], five isolates of *Enterococcus* spp. (including vancomycin-resistant *Enterococcus faecium*), six isolates of *Escherichia coli* [including extended-spectrum b-lactamase-producers], two *Klebsiella pneumoniae*, one *Serratia marcescens* AmpC-producer, four *P. aeruginosa*, one *Acinetobacter lwoffii*, one *Propionibacterium acnes*, one *Bacteroides fragilis*, two *Candida albicans*, one *Candida glabrata* and one *Aspergillus fumigatus* were tested against SurgihoneyRO™.
Control NCTC strains were also tested.

2.3. Agar diffusion

Wells of 6 mm were cut in Iso-Sensitest agar (Oxoid Ltd., Basingstoke, UK) that had already been inoculated with the test organism at a concentration to give semiconfluent growth. Test samples of SurgihoneyRO™ and other honeys in the pilot study were added to the wells.

A pilot study was carried out initially to compare SurgihoneyRO™ potencies 1, 2 and 3 with a variety of honeys from around the world (Europe, South America, New Zealand, Yemen, Sudan) and with medical honey (Medihoney[1]; Comvita, Maidenhead, UK) as well as with antimicrobial dressings containing silver (Aquacel[1] Ag; ConvaTec, Deeside, UK) and iodine (Iodoflex™; Smith & Nephew, UK). Wells were cut in plates inoculated with *S. aureus* and were filled with test honey or, in the case of the dressings, these were cut to 2 cm × 2 cm and were placed on the surface of the inoculated plates.

Following the pilot studies, SurgihoneyRO™ potencies 1, 2 and 3 were tested alone against a range of bacterial isolates from skin lesions. The wells were filled to the surface with a preparation of ca. 2 g of neat SurgihoneyRO™ of the three potencies, diluted and emulsified in an equal volume of sterile water. Zone sizes were measured after 18–24 h of aerobic incubation (72 h for *Candida* and *Aspergillus* spp., and 18–24 h anaerobically for *Propionibacterium* and *Bacteroides* spp.).

2.4. Minimum inhibitory concentrations and minimum bactericidal concentrations

SurgihoneyRO™ product was warmed to 37 8C to liquefy it and 5 g was mixed with 10 mL of sterile deionised water. This dilution was regarded as the 'neat' substance for serial dilution. The British Society for Antimicrobial Chemotherapy (BSAC) method for determining MICs and MBCs was used [16]. SurgihoneyRO™ was serially diluted in 96-well microtitre trays. Starting with neat product, 75 mL of each honey dilution was added to the next well in the strip of the microtitre tray. The neat concentration represented a concentration of 256 g/L, and the 1:2048 dilution represented ca. 0.12 g/L.

Test organisms were prepared by taking four morphologically identical colonies for each organism from pure culture to create a 0.5 McFarland standard density. This was further diluted 1:10.

All wells, including controls, were inoculated with 75 mL of the test isolate preparation and the well trays were incubated at 37 8C for 18 h. The MIC was regarded as the most dilute well that showed no detectable turbidity.

The MIC well and those around the MIC well were subcultured on blood agar (Oxoid Ltd.) and were incubated at 37 8C for 18 h to determine the MBC. The MBC was the most dilute concentration that showed no growth after incubation.

Therapeutic concentrations of SurgihoneyRO™ for comparison with the MIC and MBC were estimated by assuming that ca. 5–10 g of SurgihoneyRO™ is applied to a wound. Local exudate will result in this being diluted in 5–10 mL of fluid. This will give an approximate local concentration of 500 g/L in contact with bacteria in the wound.

2.5. Time–kill curves

The test organism inoculum was prepared by taking 0.1 mL of a 0.5 McFarland standard density of the test organism and inoculating this in 4 mL of nutrient broth. The test inoculum was divided into four separate bijous, a control and three test preparations to which were added 0.5 g of SurgihoneyRO™ 1, SurgihoneyRO™ 3 or Medihoney. Colony counts of the inocula were determined by serial dilution 1:10 and plating 0.1 mL on a blood agar plate, repeated three times.

The test and control inocula were kept at 30 8C to simulate the temperature of a superficial skin lesion. Colony counting was performed as above in triplicate at 0.5, 2, 4, 24, 48, 72 and 168 h.

A terminal culture was performed by inoculating 0.1 mL of the original inoculum into nutrient broth to neutralise any residual effect of the SurgihoneyRO™ and incubating for 72 h at 37 8C, before plating on blood agar to determine test organism survival.

3. Results

3.1. Inhibition zone sizes

The pilot comparative studies demonstrated that all of the SurgihoneyRO™ potencies had greater antimicrobial activity than any other honey tested, including the medical grade honey, Medihoney. The inhibitory zones for SurgihoneyRO™ 1, 2 and 3 were larger than those produced by any other honey. Silver dressings produced some inhibitory effect beneath the dressing but there was no zone of inhibition as there was for SurgihoneyRO™. Iodine dressings produced a large zone of inhibition (ca. 70 mm) to *S. aureus*,

Table 1
Inhibitory zone sizes with Medihoney and different potencies of SurgihoneyRO™ (S1–3).

Organism (no. of strains)	Mean (range) inhibitory zone size (mm)			
	S1	S2	S3	Medihoney
Meticillin-susceptible *Staphylococcus aureus* (12)	36.2 (32–38)	53.4 (44–58)	66.5 (60–72)	23.2 (20–25)
Meticillin-resistant *S. aureus* (6)	35.6 (31–38)	52.6 (48–59)	67.3 (59–73)	22.8 (20–24)
b-Haemolytic streptococci (6)	40.0 (35–42)	44.5 (38–51)	59.2 (53–69)	21 (18–23)
Enterococcus spp. (5)	38.0 (34–39)	49.5 (44–55)	61.8 (59–64)	19.4 (18–21)
Escherichia coli (6)	33.4 (30–37)	49.5 (36–55)	62.7 (59–69)	20 (18–23)
Klebsiella pneumoniae (2)	34.2 (30–38)	40.0 (38–42)	57.0 (52–62)	20 (17–23)
Pseudomonas aeruginosa (4)	25.8 (20–28)	34.8 (30–38)	50.2 (46–51)	17.3 (16–19)
Acinetobacter lwoffii (1)	32.1	43.7	55.2	19
Bacteroides fragilis (1)	22.3	28.7	34.2	18
Propionibacterium acnes (1)	19.7	23.4	31.9	16
Candida spp. (3)	9 (8–10)	15 (15)	26 (24–28)	0
Aspergillus fumigatus (1)	8	12	18	0

larger than SurgihoneyRO™ 1 (36 mm) and equivalent to SurgihoneyRO™ 3 (67 mm).

In the quantitative zone size testing, SurgihoneyRO™ at all potencies produced an inhibitory zone in agar diffusion against all bacteria tested, both Gram-positive and Gram-negative bacteria including MDR bacteria and fungal species. The zone size for each species increased with increasing potency of SurgihoneyRO™ preparations (Table 1). The inhibitory effect of SurgihoneyRO™ was not dependent only on direct contact with the active agent as with the silver dressings, but diffused well beyond the well producing the extensive zones listed in Table 1.

3.2. Minimum inhibitory concentrations and minimum bactericidal concentrations

SurgihoneyRO™ demonstrated potent antimicrobial activity against all of the isolates tested. MICs and MBCs were very consistent amongst isolates of the same species whether the isolates were MDR or highly sensitive. Table 2 shows the MICs and MBCs for SurgihoneyRO™ and Medihoney. The degree of potency rose with the grade of SurgihoneyRO™. The MBC for each isolate was close to the MIC, within a single dilution in most cases.

The topical concentration of SurgihoneyRO™ in wounds is estimated at ca. 500 g/L. The MIC/MBC for *S. aureus* was 32/125 g/L for SurgihoneyRO™ 1 and 0.12/0.25 g/L for Surgihoney 3RO™.

3.3. Time–kill curves

SurgihoneyRO™ kills bacteria rapidly. Starting with an inoculum of ca. 10^5 CFU/mL, numbers in the control rose steadily, whereas in the Surgihoney inocula the numbers fell rapidly after contact with both potencies of SurgihoneyRO™ (1 and 3). By 30 min, CFU numbers had fallen 1000-fold in most cases for both SurgihoneyRO™ 1 and SurgihoneyRO™ 3 (Fig. 1). For SurgihoneyRO™ 1, bacterial growth was undetectable by 2 h in most cases and for SurgihoneyRO™ 3 by 30 min. Enterococci appeared more resilient and persisted for 48 h. Cidal activity was complete for all organisms, as the terminal culture in nutrient broth with subsequent plating on blood agar failed to detect any organism in the SurgihoneyRO™ 1 or SurgihoneyRO™ 3 inocula.

4. Discussion

SurgihoneyRO™ is a natural honey that is also organic in the current sense of the word in that it has no agricultural additives or antimicrobial residues, unlike much commercial honey for human consumption. It is not dependent on particular nectar sources, unlike honeys such as manuka which depends on a specific plant nectar source for its enhanced activity. SurgihoneyRO™ has undergone a process whereby its natural antimicrobial activity has been enhanced and, for this reason, SurgihoneyRO™ is described as engineered. Antimicrobial activity can be controlled in SurgihoneyRO™ by the preparation process, allowing the production of different grades with measured potency that is consistent.

This study has clearly demonstrated the efficacy of SurgihoneyRO™ as a highly potent antimicrobial active against all species of bacteria and fungi tested. In the comparison of SurgihoneyRO™ with a variety of honeys sourced from around the world as well as with medical grade honey (Medihoney), SurgihoneyRO™ demonstrated greater antimicrobial activity. By comparison with the commonly used topical antiseptics silver and iodine, SurgihoneyRO™ 3 produced an antimicrobial effect as great as iodine dressings and greater than silver dressings (Aquacel Ag), which was only effective at inhibiting bacteria in direct contact with the dressing.

Table 2
Minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) (in g/L) of Medihoney and different potencies of SurgihoneyRO™ (S1–3).

Organism	Medihoney		S1		S2		S3	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
MSSA	64	>256	32	128	8	16	0.12	0.25
MRSA	128	>256	16	64	8	16	0.12	0.25
Group B *Streptococcus*	32	128	4	16	4	4	0.25	1
Group A *Streptococcus*	32	128	8	16	2	4	0.25	0.5
Enterococcus spp.	128	>256	32	125	8	64	1	4
Escherichia coli	128	256	32	64	4	4	1	2
ESBL-producing *E. coli*	128	256	32	128	4	4	1	2
AmpC-producing *Serratia marcescens*	128	>256	32	64	16	64	1	2
Klebsiella pneumoniae	128	256	32	128	8	8	1	2
Pseudomonas aeruginosa	128	>256	16	16	4	16	1	4
Candida albicans	>256	>256	>256	>256	16	16	4	4

MSSA, meticillin-susceptible *Staphylococcus aureus*; MRSA, meticillin-resistant *S. aureus*; EBSL, extended-spectrum b-lactamase.

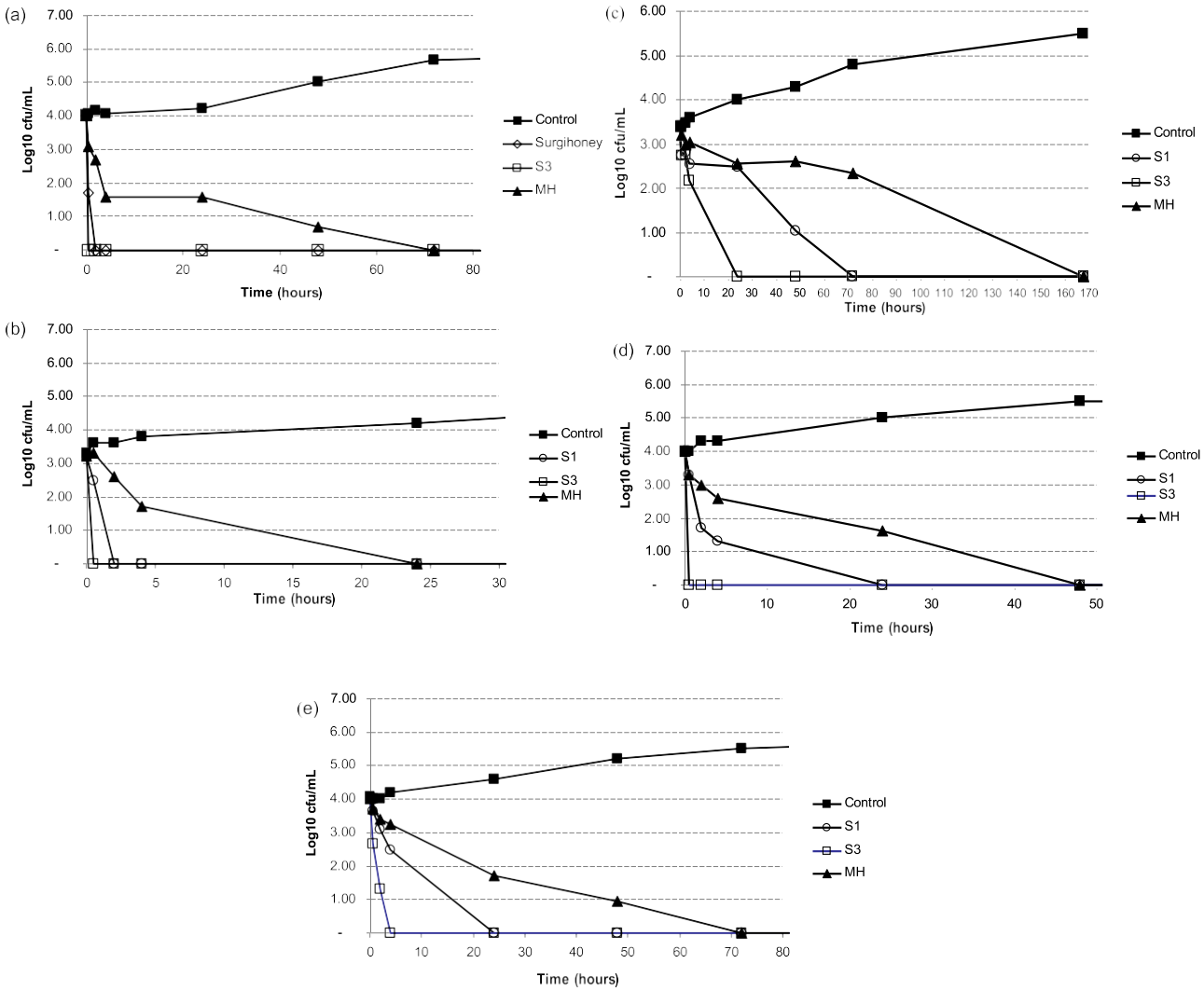

Fig. 1. Time–kill series for (a) *Staphylococcus aureus*, (b) meticillin-resistant *S. aureus*, (c) vancomycin-resistant *Enterococcus faecium*, (d) *Escherichia coli* and (e) *Pseudomonas aeruginosa*.

MIC and MBC testing show that SurgihoneyRO™ not only inhibits but also kills microbes at concentrations 10- to 1000-fold below those that are likely to be achieved in topical treatment, estimated at 500 g/L. The cidal activity of SurgihoneyRO™ occurs at concentrations close to its inhibitory activity. There is therefore the potential for SurgihoneyRO™ to be highly active in polymicrobial inhibition and eradication when applied topically in any colonised or superficially infected wounds or soft tissue cavities. As many chronic wounds are colonised with resistant bacteria [8–10], and bacterial persistence in biofilm production delays wound healing [12,13], SurgihoneyRO™ use may help to reduce inappropriate use of antibiotics as well as promote wound healing. In clinical use, topical SurgihoneyRO™ concentrations at the site of the wound will be considerably higher than those for systemic antibiotics in serum or deep tissue. This is reflected in the MIC and MBC values for SurgihoneyRO™, which are correspondingly higher than those gener- ally expressed for systemic antibiotics.

The speed of cidal activity is shown by the time–kill curves to be extremely rapid, within 30 min for SurgihoneyRO™ 3 and within 2 h for SurgihoneyRO™ 1. This is the case both for Gram-positive and Gram- negative organisms, although enterococci appear slightly more resilient. Fungi, *Candida* and *Aspergillus* spp. also require higher concentrations and more prolonged exposure to inhibit growth and kill the organism. SurgihoneyRO™ showed a more rapid bactericidal effect than Medihoney.

SurgihoneyRO™ is formulated as a sterile product to be applied as a topical wound dressing to skin lesions and cavities with the aim of providing a moist wound healing environment while also reducing microbial colonisation, helping to remove slough and to promote granulation and epithelialisation.

Other antimicrobial preparations are available as topical preparations intended to treat or prevent wound infections. Silver-impregnated dressings appear to possess good antimicrobi- al activity [17], however they also display cytotoxicity compared with honey preparations [18,19]. Iodine analogues also possess good antimicrobial activity [20] but they also have been reported to be toxic in certain situations [20–26]. There is also increasing concern about the use of chlorhexidine preparations in wound dressings owing to the development of antimicrobial resistance and toxicity [27,28].

A Cochrane Collaboration report stated that honey might be superior to some conventional dressing materials [29], but there is considerable uncertainty about the replicability and applicability of this evidence. This study has demonstrated that SurgihoneyRO™, in which the natural effect of honey is enhanced and is controllable, is superior in antimicrobial effect to currently available pharmaceu- tical honey.

The clinical utility of SurgihoneyRO™ is likely to be in topical application, on skin and in wounds and cavities. Wounds may become colonised with bacteria that can form biofilms and delay

healing [8–13]. With increasing concern about antimicrobial resistance and the lack of novel antimicrobial agents [30], a topical agent with broad antimicrobial activity could play a role in reducing the use of systemic antibiotics in soft tissue lesions. These in vitro studies have demonstrated the potential of SurgihoneyRO™ as a wound dressing with high antimicrobial activity whose potency can be controlled and that also delivers other important functions in wound healing (moist barrier, desloughing, local nutrient supply, local immune modulation [1]) and shows no toxic side effects [13,15].

In conclusion, these in vitro results support the clinical use of SurgihoneyRO™ as a wound dressing and this may be the first product that can deliver all the required roles in the healing process of wounds as well as being a potent and non-toxic antimicrobial.

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Competing interests

None declared.

Ethical approval

This was a laboratory investigation; the Research and Development Committee of Hampshire Hospitals NHS Foundation Trust (Winchester, UK) approved the study, but further ethical approval was not sought.

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