In vitro study of the effect of wound dressings on planktonic and biofilms from diabetic foot isolates

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In vitro study of the effect of wound dressings on planktonic cells and biofilms from diabetic foot infections

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Background

The diagnosis and treatment of biofilm-associated infections found in diabetic foot ulcers and other chronic wounds are challenging; mostly due to laborious diagnosis techniques and antibiotic resistance pathogens. The investigation of biofilms therefore needs a more revolutionised approach in order to alleviate their pathological effect and reduce cost to the NHS. This includes the use of alternate treatment options such as honey and silver impregnated dressings in addition to antibiotics.

Silver impregnated dressings reduce the ability of bacterial cells to adhere to each other and host cells thereby destabilizing the biofilm matrix by disrupting intermolecular forces.[1,3] Honey on the other hand, is an antimicrobial agent with some bioactive properties including, antibacterial effect, high osmolality, antioxidant activity, debriding action and enhanced rate of healing. [3,4,5]

Aim of Study

The aim of this current study was to determine the antimicrobial effect of honey-impregnated (Medihoney™ Apinate) and silver-impregnated (Acticoat and Silvercel) wound dressings on planktonic cells and biofilms of *Staphylococcus aureus* and *Proteus mirabilis.*

Methods

In this study, bacteria were grown using the conventional 6-well plate and standard agar techniques. In the 6-well plate assay, a bacterial suspension of 10^8 colony forming unit (CFU)/mL was inoculated on each dressing in excess Luria-Bertani and Muellar Hinton broths and incubated at 35 – 37° C for 30 and 60 minutes and 24 hours.[3] Atrauman dressing (with no antibiotic properties) was used as a positive control. Bacteria, were recovered in sodium thioglycolate solution (STS) after incubation, vortexed and their optical densities (OD at 600 nm) and CFU/mL determined using a 96-well microtitre plate and Mueller Hinton agar (MHA) respectively.[3, 5]

To determine the effect of the wound dressings on quasi-biofilms, 1 mL of each microorganism was pre-inoculated on MHA plates. After 30 minutes, circular shaped dressings were placed in the middle of the plates and incubated overnight at 37° C after which their zones of inhibition (ZOI) were measured.[5] Results for OD_{600} are presented as means (±SEM) at 95% confidence interval. *p* value was calculated using Student's *t.* test

Results

In the inhibition assay, none of the dressings was significantly effective ($p > 0.05$) to inhibit bacterial growth or biofilm formation at all the times tested (Figure 3).

However, Acticoat and Silvercel inhibited >50% of bacterial growth after 30 mins of incubation. Medihoney™ Apinate also inhibited >50% of bacterial growth after 24 hours of incubation (Table 1.).

In the biofilm inhibition test, Acticoat and Medihoney™ apinate produced ZOI between 1.5 – 15 mm against both *S. aureus* and *P. mirabilis* (Figure 1 A and C).

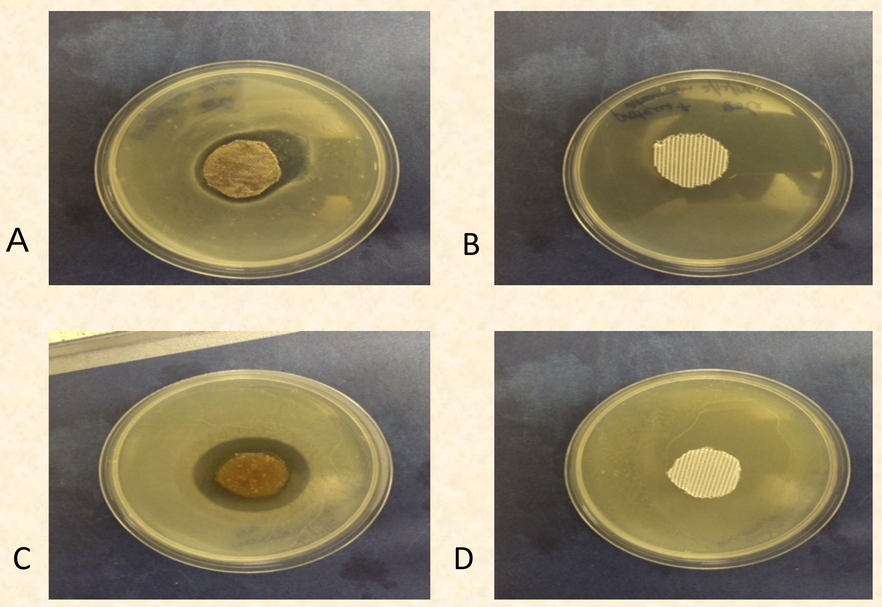

Figure 1.*P. mirabilis* and *S. aureus* quasi-biofilms grown on MHA in the presence of **A.** Acticoat ; **B**. Atrauman; **C**. Medihoney; and D. Atrauman (no ZOI) .

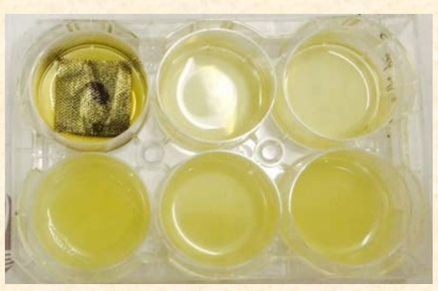

Figure 2. An inhibition test using Acticoat in a 6-well plate inoculated with *P. mirabilis.*

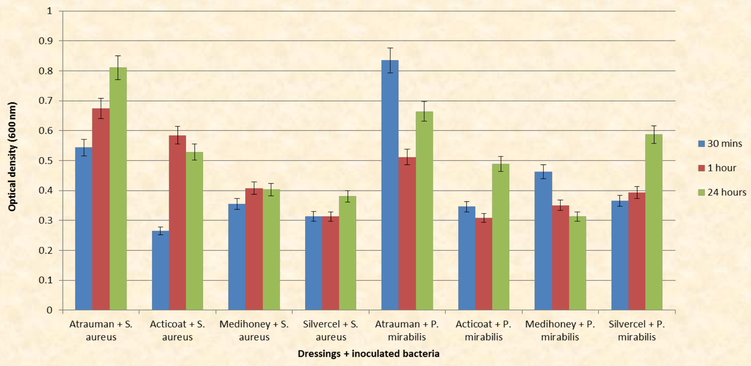

Figure 3. Inhibition of *P. mirabilis* and *S. aureus* biofilm formation by Acticoat, Medihoney and Silvercel dressings over 3 time periods – 30 mins, 1 hour and 24 hours. Atrauman was used as a positive control. The degree of inhibition for the time period tested did not correlate with each other and found to be statistically insignificant as determined by One-way ANOVA ($p > 0.05$).

	Percentage inhibition (%)		
Bacterial Isolate + dressings	30 mins	1 hour	24 hours
Acticoat + *S. aureus*	51.3	13.43	34.57
Medihoney + *S. aureus*	33.33	38.81	51.62
Silvercel + *S. aureus*	42.59	53.73	53.09
Acticoat + *P. mirabilis*	57.83	39.22	25.76
Medihoney + *P. mirabilis*	44.58	31.37	53.03
Silvercel + *P. mirabilis*	55.42	23.53	10.61

Table 1. Percentage inhibition of *P. mirabilis* and *S. aureus* after 30 mins, 1 hour and 24 hours. Results for Acticoat, Medihoney and Silvercel were compared with Atrauman (positive control).

Discussion

Though all the dressings inhibited >50% of bacterial growth at different incubation times, none of them was significantly effective to inhibit biofilm formation.

However, Medihoney™ Apinate was found to have sustained activity against both *S. aureus* and *P. mirabilis* even after 24 hours of application. This is due to the prolong bioavailability of manuka honey, its active component.[2, 4] Medihoney ™ Apinate and Acticoat were the most effective in inhibiting *S. aureus and P. mirabilis* biofilms respectively.

Previous studies have shown that some silver dressings were successful at killing or inhibiting some biofilms using atomic force microscopy.[1] Newman *et al*. (2006) demonstrated that silver salt-containing Hydrofiber (SCH) dressings killed planktonic cells of *Pseudomonas aeruginosa* 20 minutes post-exposure, and subsequently all planktonic bacteria after 100 minutes contact time.[6] It was also reported that SCH dressings showed anti-biofilm activity against bacterial biofilms after 3 hours of exposure and subsequently eradicated >90% following a 24-hour contact time.[6]

As observed in this study, it can be suggested that, dressings augmented with antibiotics can reduce chronic wound biofilms.

References

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