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Original Article

A Novel Bioengineered Wound Product with In Vitro Capabilities to Reduce Bacteria

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Abstract

Introduction: When microorganisms colonize a wound, there is a continuum which extends from contamination, to colonization and infection preventing a wound from healing. Biomaterials that reduce bacteria within the wound microenvironment can help progress it towards healing. **Objective:** This study aims to determine the *in vitro* bacteria reduction capabilities of a novel, commercially available bioengineered wound product (BWP) – a synthesis of gelatin (a highly purified collagen derivative), Manuka honey, and hydroxyapatite. **Materials and methods:** Using a direct contact method, the BWP and negative control were inoculated with suspensions of gram-positive (*Staphylococcus aureus*) and gram-negative (*Pseudomonas aeruginosa*) bacteria. After incubation for 30 minutes and 24 hours, recovery of viable organisms was performed. **Results:** There was a significant reduction (99.99%) in bacterial load recovered from the BWP at 24 hours compared to the negative control. Additionally, the BWP caused a significant reduction in bacterial load at 24 hours compared to 30 minutes (97% and 64% reduction for *Staphylococcus aureus* and *Pseudomonas aeruginosa,* respectively). **Conclusions:** The significant reduction in bacterial load *in vitro* suggests that the product has the potential to help manage the pathogenic bioburden of a wound..

Introduction

When microorganisms colonize a wound, there is a continuum which extends from contamination, to colonization and infection preventing a wound from healing. *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus* (MRSA) and Pseudomonas aeruginosa are the predominant microbial strains that occur in patients with infected wounds [1]. Biomaterials that reduce bacteria within the wound microenvironment can help progress it towards healing. Compounds such as Manuka honey, silver, gold, and zinc have been integrated into wound dressings to achieve therapeutic effects on bacteria [2]. Specifically, Manuka honey is an attractive and natural material that has been shown to inhibit bacterial growth [3,4]. In this study, an absorbable novel bioengineered wound product (BWP), a synthesis of gelatin (a highly purified collagen derivative), Manuka honey and hydroxyapatite, was evaluated for *in vitro* bacteria reduction capabilities against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. While Manuka honey has been traditionally used for topical, short-term dressing applications, this novel BWP uniquely synthesizes Manuka honey into a longer-lasting, fully absorbable solid sheet for managing wounds.

Materials and methods

Preparation of the BWP and control product

The novel BWP (APIS®, SweetBio, Inc., Memphis, TN) was prepared aseptically to 2 x 2 cm sections. Each sample was immersed in sterile saline solution for one minute, turned over, and immersed in sterile saline for a further minute to ensure the BWP was fully hydrated. This preparation procedure was also replicated for the bacterial barrier negative control dressings (N-A® Knitted Viscose Dressing).

Bacteria reduction assessment via direct contact method

Suspensions of both gram-positive (Staphylococcus aureus ATCC® 6538™) and gram-negative (Pseudomonas aeruginosa ATCC® 9027™) bacteria were prepared to 1 x 10^6 ± 5 x 10^5 $CFUmL^{-1}$ in Tryptic Soy Broth (Scientific Laboratory Supplies, UK). The inoculum was enumerated by performing 10-fold dilutions and plating out the resulting suspensions onto Tryptic Soy Agar (TSA, Scientific Laboratory Supplies, UK). One hundred microliters of 1 x 10^6 ± 5 x 10^5 $CFUmL^{-1}$ bacterial suspension was used to inoculate the BWP and control samples. Samples were incubated for 30 minutes and

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24 hours at 37 ± 2°C. Following each incubation period, the BWP and control samples were placed into 3 mL of Quench neutralizing solution (Tween 80 30 g/L, Lecithin 30 g/L, L-Histidine 1 g/L and Sodium thiosulphate 4 g/L in distilled water (pH 7)) and sonicated using a sonicating water bath (VWR, UK) for five minutes to recover any viable organisms. Microorganisms were enumerated by performing 10-fold dilutions of the resultant suspensions and plating onto TSA in duplicate. All tests were performed in triplicate.

Statistical analysis

Average Log10CFUmL^{-1} bacterial recoveries and average Log10CFUmL^{-1} reductions, compared to the negative control, are presented as mean ± standard deviation (SD) from three independent replicates, with duplicate repeats for each independent replicate. The minimum limit of detection for this study was 1 Log. A Student's unpaired t-test (two-tailed) was used to assess statistical differences between the Log10CFUmL-1 recovery data from the negative control and BWP samples. Data was considered statistically significant when $p < 0.05$.

Results

Staphylococcus aureus

Following 24 hours incubation, the quantity of bacteria recovered from the negative control rose from 4.61 ± 0.04 Log10CFUmL^{-1} (30 minutes) to 6.85 ± 0.19 Log10CFUmL^{-1} (24 hours) while the quantity of viable material recovered from the BWP reduced from 4.49 ± 0.03 Log10CFUmL^{-1} to 2.89 ± 0.19 Log10CFUmL^{-1} (Figure 1). This was an average reduction of 99.99% (3.96 ± 0.19 Log10CFUmL^{-1} reduction) compared to the negative control at 24 hours ($p < 0.001$).

Pseudomonas aeruginosa

Following 24 hours incubation, the quantity of bacteria recovered from the negative control rose from 4.83 ± 0.10 Log10CFUmL^{-1} (30 minutes) to 8.42 ± 0.05 Log10CFUmL^{-1} (24 hours) while the quantity of viable material recovered from the BWP reduced from 4.65 ± 0.04 Log10CFUmL^{-1} to 4.21 ± 0.03 Log10CFUmL^{-1} (Figure 1). This was an average reduction of 99.99% (4.22 ± 0.03 Log10CFUmL^{-1} reduction) compared to the negative control at 24 hours ($p < 0.001$).

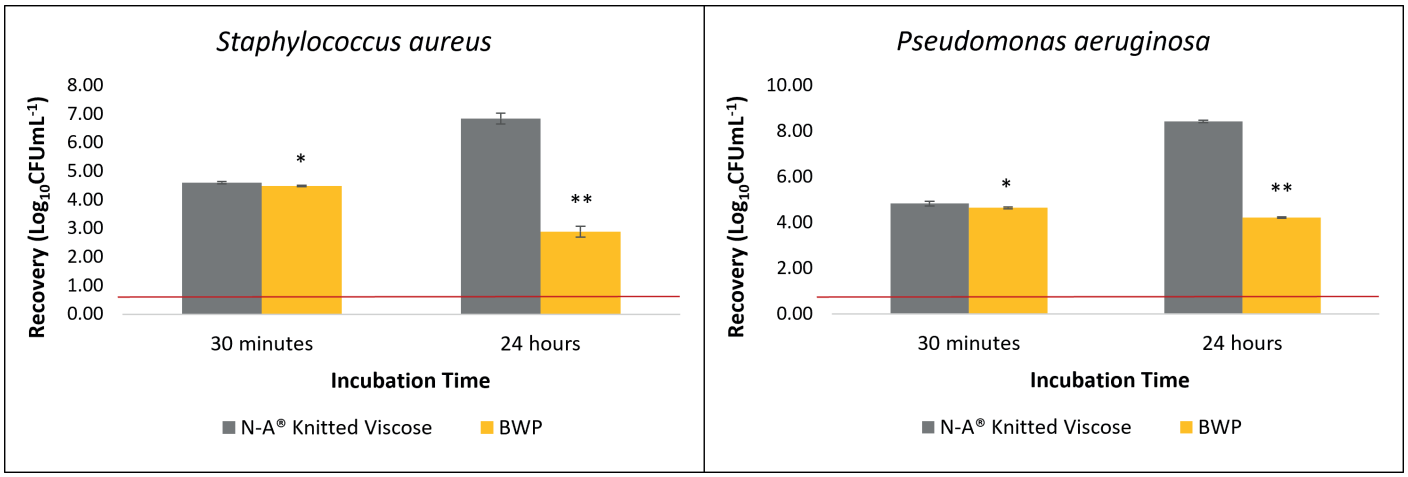

Figure 1. Quantity of viable *Staphylococcus aureus* (left) and *Pseudomonas aeruginosa* (right) recovered from the BWP and control items after 30 minutes and 24 hours incubation. The error bars indicate the standard deviation of the mean. The red line represents the limit of detection (1 Log). * = $p < 0.05$, ** = $p < 0.001$.

Table 1. Average recovery of viable *Staphylococcus aureus* and *Pseudomonas aeruginosa* following 30 minutes and 24 hours treatment with the BWP, compared to the negative control. CFU = colony forming units, SD = standard deviation, N/A = not applicable. * = $p < 0.05$, ** = $p < 0.001$.

Organism	Test Item	Average Log Recovery (Log10CFUmL^{-1} ± SD)		Average Log Reduction (Log10CFUmL^{-1} ± SD)	
		30 minutes	24 hours	30 minutes	24 hours
Staphylococcus aureus	N-A® Knitted Viscose	4.61 ± 0.04	6.85 ± 0.19	N/A	N/A
	BWP	4.49 ± 0.03	2.89 ± 0.19	0.12 ± 0.03*	3.96 ± 0.19**
Pseudomonas aeruginosa	N-A® Knitted Viscose	4.83 ± 0.10	8.42 ± 0.05	N/A	N/A
	BWP	4.65 ± 0.04	4.21 ± 0.03	0.18 ± 0.04*	4.22 ± 0.03**

Discussion and Conclusion

Between 30 minutes and 24 hours, the bacterial load recovered from the N-A® Knitted Viscose negative control dressing increased, demonstrating that bacterial growth was supported by this product. A significant reduction (99.99%) in bacterial load recovered from the BWP at 24 hours compared to the negative control at 24 hours demonstrated, at minimum, bacteriostatic properties of the BWP. In addition, the BWP caused a significant reduction in bacterial load at 24 hours compared to 30 minutes (97% and 64% reduction for *Staphylococcus aureus* and *Pseudomonas aeruginosa*, respectively), demonstrating a slight antibacterial impact of the BWP. The significant reduction in bacterial load *in vitro* suggests that the product has the potential to help manage the pathogenic bioburden of a wound. Future *in vitro* studies will assess if the BWP has the potential to be antimicrobial or serve as a microbial barrier.

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Conflict of interest

I.R. and A.S. are executives of SweetBio, Inc. I.R. is a co-inventor of the BWP technology. All other authors have no conflict of interest to declare. The content of this article was expressly written by the authors listed. No ghostwriters were used to write the article.

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