A Novel *In Vitro* Assay for Evaluating the Malodor Inhibition Properties of Wound Dressings Containing Odor Abatement Technologies

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INTRODUCTION

Offensive odor emanating from a wound is indicative of bacterial colonization and/or necrotic tissue within the wound.[1,2,3] Metabolic byproducts produced by bacteria as well as the breakdown of tissue result in the release of volatile compounds that cause the unpleasant odor. While malodor itself is not harmful to patients, it can be unpleasant and distracting to the patient, relatives, and caregivers. Additionally, the presence of malodor can also be a cause of embarrassment and distress for the patient. Consequently, a number of wound dressings are commercially available that help to manage the wound environment to promote healing and contain odor abatement (OA) technologies to mitigate malodors. These technologies act by either killing the bacteria that cause the malodor using antimicrobial agents or they adsorb/capture the volatile compounds responsible for the malodor. The mode-of-action (MOA) by which odor reduction occurs can significantly impact how the FDA and other regulatory bodies classify the device, therefore being able to differentiate the MOA is critical. The novel *in vitro* assay utilized in this study was designed to quantitatively demonstrate odor reduction and differentiate the MOA of the OA technology.

Until recently, a standardized, quantitative malodor inhibition assay did not exist to assess OA technologies used in wound dressings. In 2021, the American Association of Textile Chemists and Colorists (AATCC) approved the first standardized bacterial-based malodor inhibition assay which can be used to evaluate the malodor inhibition exhibited by textiles treated with an odor controlling/capture technology and/or an antibacterial technology. The test method is AATCC Test Method 211-2021 and it was designed specifically to test textiles and was incapable of testing anything that laid flat or a specific surface/side of a multi-component material (i.e., wound dressings)[4]. While this test method focused on testing textiles samples, it provided a useful template from which we were able to modify it to test wound dressings in a simple, simulated end-use environment.

MATERIALS & METHODS

The test apparatus used in Biodaptive's BBMI assay allows for the evaluation of thicker materials, multi-layered materials, and/or assessment of microbial contamination of a specific side of the sample, which was not possible with AATCC TM 211-2021. Additionally, at the end of the exposure period the test samples from Biodaptive's BBMI assay could easily be recovered and the number of viable bacteria that survived could be enumerated, which is not possible with the AATCC TM 211-2021.

During Biodaptive's BBMI assay, the test sample (a wound dressing) is inoculated on the patient contacting surface with a solution containing nutrients, urea, and a known concentration of bacteria (5.38 Log_{10} CFU/mL). *Proteus vulgaris* (ATCC 29905), a gram-negative bacterium, was selected as the bacteria for this assay due to this organism's ability to metabolize urea and protein residues to produce ammonia, the surrogate malodor evaluated in this assay. Ammonia is a component of bacterial malodor and can be easily and inexpensively detected using commercially available Dräger gas detector tubes.

After the inoculum was fully absorbed by the test sample, the sample is placed into the base of a 50mm polystyrene petri dish, with the patient contacting surface facing down. A plastic O-ring was placed on top of the dressing around the edge of the apparatus to help seal the device. A polystyrene petri dish lid was modified with an opening in the center of the lid and an adapter was used to attach a Dräger gas detector tube to this opening. The lid was then placed on top of the petri dish base. The petri dish base and lid were sealed with Parafilm. A Dräger gas detector tube was then inserted in the adapter on the petri dish lid and sealed with Parafilm. The assembled test apparatus (see Figure 1) was then incubated at 36°C and observed at regular intervals for the detection of ammonia.

In this test, the bacteria located on the patient contacting surface layer of the dressing sample converts urea and protein residues into ammonia. Due to the volatile nature of ammonia coupled with its low density, ammonia will migrate through the various layers of the dressing sample to the opposite side of the dressing. At this point, any ammonia not adsorbed/captured by the dressing is collected in the headspace of the test apparatus and measured using a Dräger gas detector tube (see Figure 2). The amount of ammonia released from the sample was observed at regular intervals, up to 8 hours, for the quantity of ammonia detected. Post-incubation, each apparatus was unsealed and the surviving bacterial populations on each sample were enumerated. The quantity of surviving bacteria recovered from each sample was compared to the initial concentration initially inoculated onto the sample to evaluate the antimicrobial activity exhibited.

Figure 1. Testing apparatus for the BBMI assay

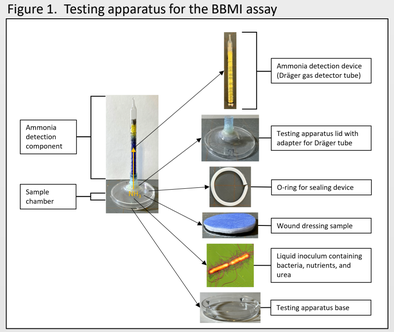


Figure 2. Color change that occurs within Dräger gas detector tube to indicate the concentration of ammonia detected

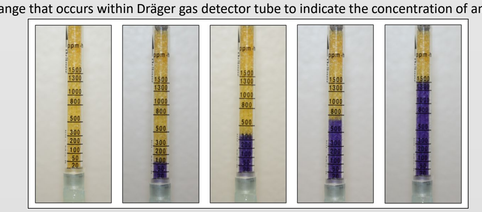


Table 1. Test samples examined in this study and a brief description of each sample

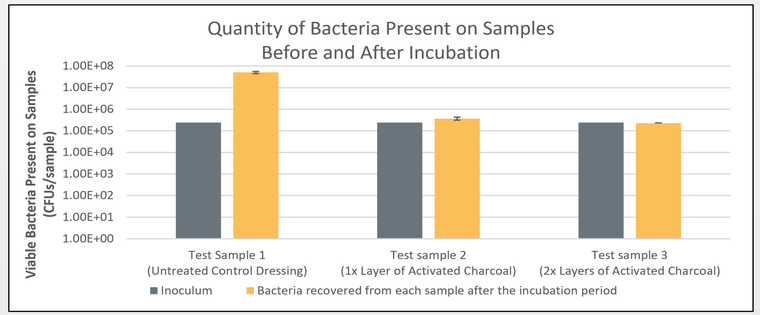
Sample	Description
Test sample 1	Untreated control dressing, no OA technology
Test sample 2	Dressing with 1x layer of non-woven material treated with activated charcoal layer as an OA technology
Test sample 3	Dressing with 2x layers of non-woven material treated with activated charcoal layer as an OA technology

RESULTS

Table 2. Magnitude of malodor reduction each sample achieved during the BBMI assay.

Sample	% Malodor Reduction (Relative to Untreated Control Dressing)
Test sample 1 (Untreated Control Dressing)	N/A
Test sample 2 (Dressing with 1x Layer of Activated Charcoal)	> 98.67%
Test sample 3 (Dressing with 2x Layers of Activated Charcoal)	> 98.67%

Figure 3. A comparison of the quantity of bacteria initially inoculated onto each sample at the beginning of the BBMI assay (t=0 hrs) and recovered from each sample at the end of the test (t=6.25 hrs).



DISCUSSION

The untreated control dressing reached the maximum value on the Dräger gas detector tubes at 6.25 hours, which was determined to be the endpoint for the test. No ammonia was detected from any of the dressings containing an OA technology, indicating that these samples exhibited a >98.67% reduction in malodor compared to the untreated control sample dressing. To demonstrate that these finding were due to the sample material adsorbing or trapping the odor, rather than the material exhibiting a bactericidal effect, the population at the beginning of the test and at the conclusion of the test were compared. None of the test samples exhibited a bactericidal effect on the test organism, so it can be concluded that the odor reduction observed with the test samples was due to the material capturing the odor and not from killing the bacteria. Consequentially, this assay may provide a means by which medical device manufacturers can demonstrate to regulatory bodies that the MOA of OA technologies incorporated into a wound dressing, or other medical device, is odor absorption/capture and not due to antimicrobial activity. This could avoid unnecessary up-classification of the device due to the perception that it contains an antimicrobial agent.

CONCLUSIONS

- Both samples containing the activated charcoal-based odor abatement technology exhibited a >98.67% odor reduction compared to the untreated control dressing.
- There was no notable reduction in the bacterial population on any of the test samples containing an OA technology, regardless of the quantity of OA technology used. Therefore, the OA technology did not function as an antimicrobial agent.
- The novel BBMI assay utilized in this study was able to quantitatively assess the malodor reduction achieved by a wound dressing containing an OA technology and clearly demonstrated that the malodor reduction MOA was due to odor capture and not a consequence of a bactericidal agent.

REFERENCES

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