A NOVAL POLYURETHINE FOAM WOUND DRESSING WITH SILVER, METHYLENE BLUE AND GENTIAN VIOLET: CONFIRMING THE PRESENCE OF SINGLET OXYGEN GENERATION AND ANTIMICROBIAL ACTIVITY

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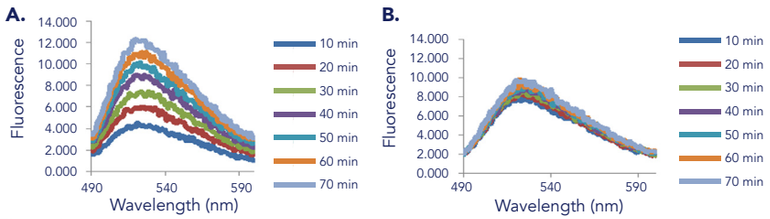


Figure 1. (Above) Overlay of fluorescence spectra of (A) methylene blue with Sensor Green and (B) Sensor Green only in DI water, after each 10-minute light exposure. The emission fluorescence spectra were measured from 490nm to 600nm, with 470nm excitation.

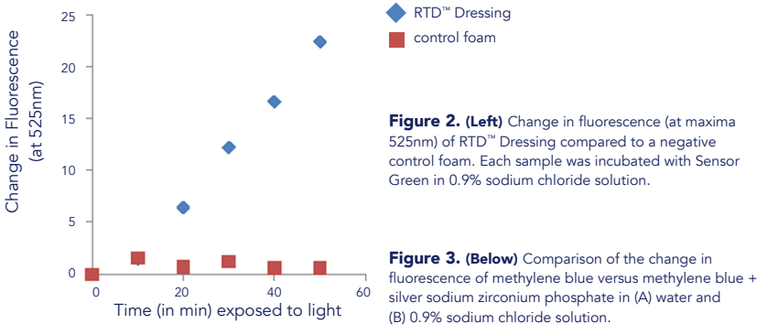
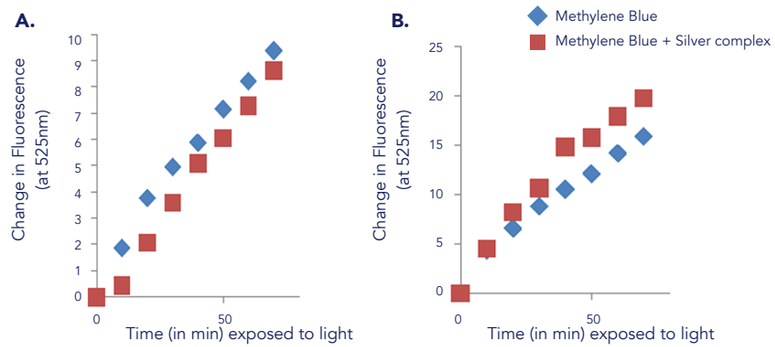


Figure 2. (Left) Change in fluorescence (at maxima 525nm) of RTD™ Dressing compared to a negative control foam. Each sample was incubated with Sensor Green in 0.9% sodium chloride solution.

Figure 3. (Below) Comparison of the change in fluorescence of methylene blue versus methylene blue + silver sodium zirconium phosphate in (A) water and (B) 0.9% sodium chloride solution.



Log_{10} Reduction	Staphylococcus aureus (ATCC 12600)	Enterococcus faecalis (ATCC 19433)	Pseudomonas aeruginosa (ATCC 10145)	Klebsiella pneumoniae (ATCC 49472)
≥ 5	1 hour	>24 hours	30 mins	24 hours
≥ 4	10 mins	>24 hours	10 mins	6 hours
≥ 3	10 mins	24 hours	10 mins	6 hours
≥ 2	10 mins	24 hours	10 mins	1 hour

Table 1. Contact time required for log reduction of four selected bacteria strains.

INTRODUCTION

Silver wound dressings have the advantage of having broad antimicrobial effectiveness against gram-negative and gram-positive bacteria[2] as well as a positive impact on wound healing[3]. Organic pigments such as methylene blue (MB) and gentian violet (GV) have also been used in wound care due to their antimicrobial, analgesic benefit and attraction for protein based chemicals. It is expected that the combination of MB, GV, Silver and the proprietary technology of integrating and tightly bounding these ingredients into a foam matrix will significantly increase the antimicrobial activity and production of Singlet Oxygen.

An independent laboratory has developed and performed assays to provide scientific evidence for the effectiveness of the RTD™ dressing and to define its mechanism of action. Two Technical Objectives were proposed to characterize the RTD™ Wound Care Dressing:

Objective 1: Designed to develop an analytical method to detect singlet oxygen generated by the formula.
Objective 2: Monitor the antimicrobial activity of the wound dressing over time.

The results of these two objectives are reported below.

METHOD – CONFIRMING THE PRESENCE OF SIINGLET OXYGEN GENERATED BY RTD™ WOUND CARE DRESSING

The detection reagent, Sensor Green (Thermo Fisher Scientific, Inc.), emits at 525nm maxima (excitation maxima 504nm) in the presence of singlet oxygen, and does not respond to the other reactive oxygen species, such as hydroxyl radical or superoxide.

Test samples containing methylene blue alone, methylene blue with silver sodium hydrogen zirconium phosphate (Alphasan RC-2000), or a section of RTD™ wound care dressing were added to deionized water (DI water) or 0.9%wt. sodium chloride solution with 1µM of Sensor Green.

The sample mixtures were placed in separate wells on a 96-well plate, and exposed to a halogen lamp (<50W/12V) at a 10 cm distance for up to 70 minutes, at 10 minute intervals. At each 10-minute interval, the fluorescence spectrum (490-600nm emission/470nm excitation) of each sample was measured by a plate reader (Varioskan LUX, Thermo Fisher Scientific).

The fluorescence emission scans of methylene blue with Sensor Green and Sensor Green only (negative control) were overlaid and shown in **Figure 1**. The fluorescence of the methylene blue (with Sensor Green) increased after each 10-minute light exposure, whereas the fluorescence of the Sensor Green only sample remained relatively unchanged after light exposure. This confirms that singlet oxygen was generated by methylene blue when exposed to light, and that the assay is effective. For comparison, the change in fluorescence (at maxima 525nm) was plotted against time (in minutes). The change in fluorescence was calculated by: Change in fluorescence = F - F0 where F is the fluorescence measured and F0 is the fluorescence measured before light exposure (time=0 mins). **Figure 2** presents the change in fluorescence over time for the RTD™ Wound Dressing compared to that of a control foam (without antimicrobial active ingredients). The data suggest that singlet oxygen was generated by the RTD™ Wound Dressing when exposed to light.

In order to measure the effect of silver sodium hydrogen zirconium phosphate on the singlet oxygen generation efficiency of methylene blue, 3.2µg/mL of methylene blue was mixed with 89.6µg/mL of silver sodium hydrogen zirconium phosphate (same weight ratio as in the RTD™ Wound Dressing formula) and Sensor Green. The change in fluorescence of the mixture was plotted and shown in **Figure 3**.

The change in fluorescence of the methylene blue-silver complex mixture over time had a minor improvement from the methylene blue sample alone.

METHOD – ANTIMICROBIAL ACTIVITY OF THE RTD™ WOUND CARE DRESSING

A previous report provided by an independent laboratory[1] has shown broad spectrum antimicrobial activity of the RTD™ Wound Dressing after 24+ hours of contact time.

The goal of our experiment is to measure the log reduction of selected bacteria at shorter contact times.

The assay was modeled after the standard Zone of Inhibition assay. Two cm by two cm cut-outs of the RTD™ Wound Dressing were soaked in 0.9% sodium chloride solution, and placed on Luria-Bertani (LB) medium agar plated with 106, 105, 104 or 103 of bacteria. The surface of the RTD™ Wound Dressing squares covering the agar plates were in contact with approximately 105, 104, 103 and 102 bacteria, depending on the total number of bacteria plated on the agar.

The wound dressing cut-outs were in contact with the agar surface for 10 minutes, 30 minutes, 1 hour, 5 hours and 24 hours at room temperature, then the agar plates were incubated at 37°C for 16 hours. A complete inhibition of growth of the bacteria where the RTD™ cut-outs were in contact with the agar plate is considered to be a log_{10} reduction of ≥5, 4, 3 or 2, depending on the number of bacteria in contact of the RTD™ cut-outs. A summary of the contact time required for log_{10} 5, 4, 3 or 2 reductions of four selected bacteria that are relevant to human infections are shown in **Table 1**.

CONCLUSION

The proposed objectives were completed and below are the key findings:

1. A fluorescence-based singlet oxygen detection method was established.
2. The generation of singlet oxygen by the RTD™ Wound Dressing was confirmed.
3. Shorter contact times required for a 5 log reduction by the RTD™ Wound Dressing were measured for Staphylococcus aureus and Pseudomonas aeruginosa, compared to previously reported data[1].

References:
1 The RTD® Advanced Wound Care Dressing, RTDWP-052014.
2) Myers BA. Wound Management. Upper Saddle River, NJ: Prentice Hall;3rd Edition 2011
3) Lo SF, Chang CJ, Hu WY, Hayter M, Chang YT. The effectiveness of silver-releasing dressings in the management of non-healing chronic wounds: a meta-analysis. Journal of Clinical Nursing. 2009 Mar; 18 (5):716-28.

RTD: RTD™ Wound Dressing (Trademark of Keneric Healthcare) Polyurethane, Silver Sodium Hydrogen Zirconium Phosphate (7 mg/gm), Methylene Blue (0.25 mg/gm) and Gentian Violet (0.25 mg/gm)