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Clinical and Laboratory Assessment for the Quantification of Biofilm and the Efficacy of Biofilm Disruption Using a Wound Gel* in Diabetic Foot Ulcers Compared to Standard of Care#: A Pilot Study

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* Next Science™ BlastX®, #Solosite

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ABSTRACT

Chronic ulcers harbor a plethora of microorganisms that are resistant not only to conventional wound care but also are resistant to physical debridement, topical therapies and dressings The presence of biofilms in chronic wounds, present in over 80% of patients with infection, and is a significant obstacle to wound closure. To date there is little-to-no science that correlates biofilm reduction in chronic wounds and healing.

The use of a novel technique to identify and quantify biofilm in chronic wound tissue is used to quantify the amount of biofilm in the wound and correlated with wound progression. Using biofilm disruptive agents that are species indiscriminate with low to no toxicity to the host tissue is the most promising approach to solving the chronicity of diabetic wounds. The objective of this 10-subject, prospective randomized pilot study is to assess the reduction in the number of viable biofilm specific microorganisms and the associated reduction in size or closure of diabetic foot ulcers treated with a biofilm disrupting wound gel compared to standard of care over 12 weeks.

In our study, we were able to quantify and differentiate planktonic and biofilm bacteria for all samples. There was a generalized trend towards a rapid reduction in bacteria and bacterial biofilm load over time of treatment. In general, as the bacterial and biofilm load decreased, the wound size was reduced.

INTRODUCTION

The National Institute of Health estimates that biofilm-based infectious disease represent up to 80% of all forms of infectious diseases[1]. The concept of biofilm and its suspected presence on the surface of chronic wounds has been investigated by the scientific community since the late 90s[2], and was evidenced by Swogger and Wolcott in 2007[3]. Using scanning electron microcopy, Swogger and Wolcott, directly demonstrated the presence of biofilm in 60% of chronic wounds compared to 6% or less in acute wounds indicating that the presence of biofilm may explain their different treatment outcomes.

For the medical community, the presence of biofilm, in chronic wounds in particular, remains often unrecognized as an important barrier to wound healing. Resistance to conventional antimicrobial treatment is not only due to the physical barrier created by the rapidly established EPS biofilm but also the expression of up to 800 new proteins secreted within hours of attachment/clustering[4].

To address the issue of resistant organisms and improve chronic wound treatment success, Next Science™ has developed a wound gel formulation based on a material science approach which targets both the biofilm and the microorganisms entrenched within. The effectiveness of Blast has demonstrated significantly higher efficacy than that of comparators in two previous clinical studies. Hence, clinical research evidence substantiates that the combination of BlastX, with standard wound debridement, significantly improves wound healing rates. Given the significant wound size reduction and closure rates observed in these short and long-term studies, as well as the lack of related serious adverse events, we aim to further understand the impact of the gel on the reduction of individual species found within the microbiome of DFUs under treatment.

OBJECTIVE

The objective of this 10-subject, prospective randomized pilot study is to assess the reduction in the number of viable biofilm specific microorganisms and the associated reduction in size or closure of diabetic foot ulcers treated with a biofilm disrupting wound gel compared to standard of care over 12 weeks.

METHODS

Clinical Methods:

Patients were randomly enrolled into the BlastX or SoloSite group. Patients were offloaded via a CAM walker and seen weekly after randomization. At weekly visits, patients were treated with sharp debridement of the wound and washing with saline (NaCl). Samples for biofilm and bacterial analysis were taken from tissue debridement. Solosite or BlastX applied Q Daily per randomization. At each visit the following assessments were performed and samples were obtained; wound assessment, debridement and biofilm sampling, wound imaging and measurements.

Laboratory Methods:

Measurement of Total bacteria

1. The curettage sample will be received in sterile 15 ml tube with 1.5 ml PBS containing 5 ppm of Tween 20.
2. Sample volume (ml) and weight (mg) will be measured
3. Sample volume adjusted to 2.0 ml using PBS + Tween 20
4. Tube and sample will be vortexed for 1 min
5. 1.6 ml of the vortexed sample will be transferred into a sterile tube for biofilm differentiation via bleach insensitivity and analysis by selective agars
6. Plate 3 x 100 µl volumes on to 3 separate TSA plates from the remaining neat solution (100 µl remain).
7. Use 100 µl of the remaining neat to create the first 1/10-fold dilution (100 µl + 900 µl).
8. Serial dilutions (1/10) of the sample solution will be performed in Maximum recovery diluent (MRD) (or similar) up until 1/1,000-fold.
9. Enumerate total bacterial numbers by plating 100µl of the appropriate dilution(s) of sample solution (in triplicate) on to non-selective agar (TSA).
10. Plates will be incubated for 48 h at 30-35ºC.
11. Following incubation, organism counts will then be recorded from each plate and dilution tested.
12. Count plates on which 15 CFU to 300 CFU have appeared, if less than 15C FU are recovered on neat plates record the actual number of colonies in the raw data and use in the calculations.. If no colonies are recovered on any of the plates from the dilution series, record the number 0 in the raw data. The detection limit should then be applied to final results.

Analysis of Biofilm Bacteria

1. Start with 1.6 ml of neat solution which was previously isolated in Section A, Step 5.
2. Add 0.8 ml 0.1% NaClO in 400 mM phosphate buffer at pH=5.8 agitate and after 10 minutes of treatment, then add 0.8 ml 0.15% Na2S2O3 in deionized water to inactivate residual NaClO and agitate.
3. The neat solution is plated in triplicate on to 8 different agars (1 non-selective, 7 selective), using 2.4 ml of the neat solution (800 µl remain).
4. Serial dilutions (10-fold, 100-fold, 1000-fold) of the neat sample solution (100 µl + 900 µl) will be performed in Maximum recovery diluent (MRD) (or similar).
5. The diluted solutions are individually plated,100 µl/plate, in triplicate on to 8 different agars (1 non-selective, 7 selective), using 2.4 ml of the diluted solution.
6. Plates will be incubated for 48 h at 30-35º C.
7. Following incubation, organism counts will then be recorded from each plate and dilution tested.
8. Count plates on which 15 CFU to 300 CFU have appeared, if less than 15 CFU are recovered on neat plates record the actual number of colonies in the raw data and use in the calculations. If no colonies are recovered on any of the plates from the dilution series, record the number 0 in the raw data. The detection limit should then be applied to final results.

RESULTS

As of this time, 8 patients have been enrolled in the study, with only 5 patients having completed the 12 weeks and/or been completely healed. As can be seen from Figure 1, the patients had decreased wound size over the treatment time, with higher initial healing rates for the BlastX group (not statistically significant).

The results for the bacteria and biofilm quantification are shown in Figures 2 and 3. As can be seen by Figure 2, there is a strong correlation between the amount of total bacteria in the wound and the amount of biofilm.

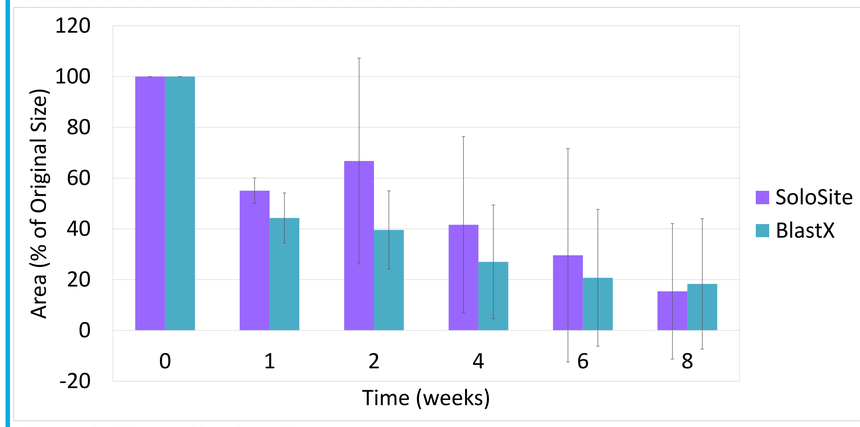

Figure 1: *Wound Size Over Time*

FUNDING

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RESULTS (continued)

From this testing, it also appears that over 4 log/mL of total bacteria has to be present in the wound for there to be a countable amount of biofilm bacteria. The results in Figure 3 seem to indicate slightly less biofilm bacteria in the Next Science samples. At this time the sample size is too small to make any statistical statements.

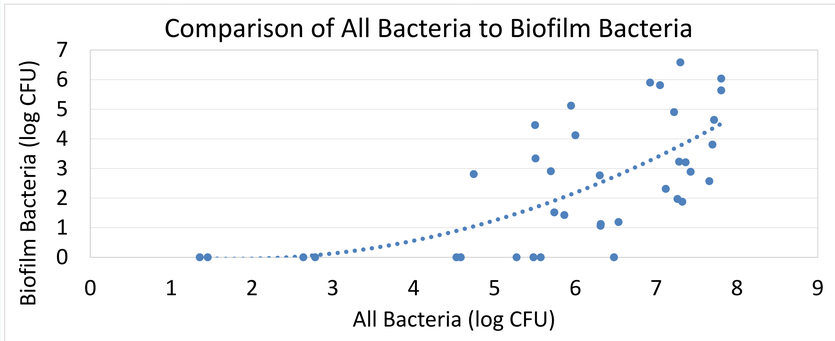

Figure 2: *Bacterial Quantification*

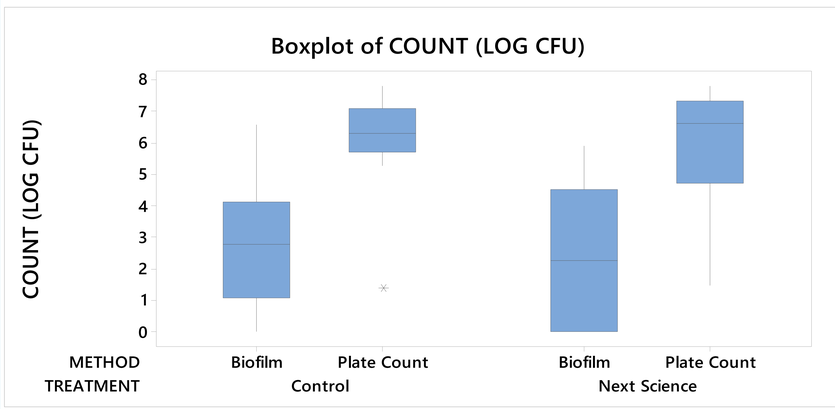

Figure 3: *Bacterial Counts by Treatment*

DISCUSSION

This is an ongoing pilot study which is intended to develop guidelines for additional testing and review trends for a larger clinical trial. Thus far, the wound measurement method is providing coherent results which are pointing towards a trend in increased healing rate with the BlastX product. The bacterial enumeration test method is providing quantifiable data for all of the bacteria and there is a trend for increased bacterial load to be indicative of increased biofilm load in the wounds. The data thus far also suggests that there must be greater than 4 log/mL of total bacteria in a wound for a biofilm to be quantifiable.

CONCLUSIONS

The purpose of this study with a small sample size is to identify trends, with the following trends having been identified.

1. Initial healing rate differences of the BlastX to Control group are not yet statistically significant.
2. It appears that a wound must have over a 4 log/ml of total bacteria in order to develop a bacterial biofilm.

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