In Vivo and *In Vitro* Evaluation of the Properties of Drawtex LevaFiber Wound Dressing in an Infected Burn Wound Model

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Introduction

Burn wounds are dynamic and complex lesions that can be challenging to treat when infected. Initial treatment with topical agents and dressings is designed to create a physical barrier against wound contamination, inhibit bacterial proliferation, provide an environment conducive to healing, and absorb exudate.[1–4]

Drawtex is a unique hydroconductive dressing that is designed to move large amounts of exudate, bacteria, and wound debris from the wound to the dressing. It has been shown in some case studies to decrease granulation, slough, and eschar from a wound bed.[5] Despite these potentially valuable features as a dressing for treating and managing infected burn wounds, Drawtex has not been tested in a controlled infection model. Further, little is known about its true measurable limitations and capacity to remove protein and cellular materials from a wound environment. The goals of this pilot study were to demonstrate and measure protein and bacterial absorption by Drawtex through the use of *in vivo* and *in vitro* models.

Materials and Methods

Burn Wound Infection Model

All animal work described herein was approved by the MedStar Health Research Institute (MHRI) Institutional Animal Care and Use Committee. Animal receipt and husbandry was provided in accordance with standard operating procedures under an animal care and use program accredited by the Association for Assessment and Accreditation of Laboratory Animal Care.[6]

Male Sprague-Dawley rats (Harlan Labs, Frederick, MD) were prepared for wound creation as described by Shupp et al.[7] Paired burn wounds were created with a 2 cm x 2 cm aluminum billet on each animal approximately 1 cm lateral to the midline on each side of the spine. Digital images were taken of both wounds, and the animals were returned to clean, sterile cages.

On post-burn day 1, the rats were anesthetized and both burn wounds were inoculated with a virulent strain of methicillin-resistant *Staphylococcus aureus* (MRSA). From a nutrient broth with 1 x 10^8 colony forming units (CFU) per ml, a 0.2 ml aliquot was applied to 2 cm x 2 cm non-adhesive gauze squares, and a gauze was then

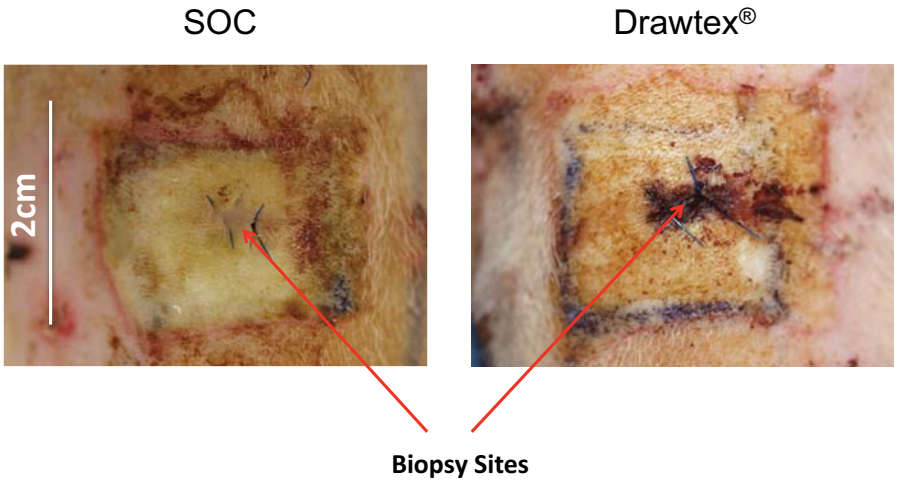

Figure 1. Representative infected burn wounds from animals treated with standard of care (left) and with Drawtex (right) on post-burn day 4.

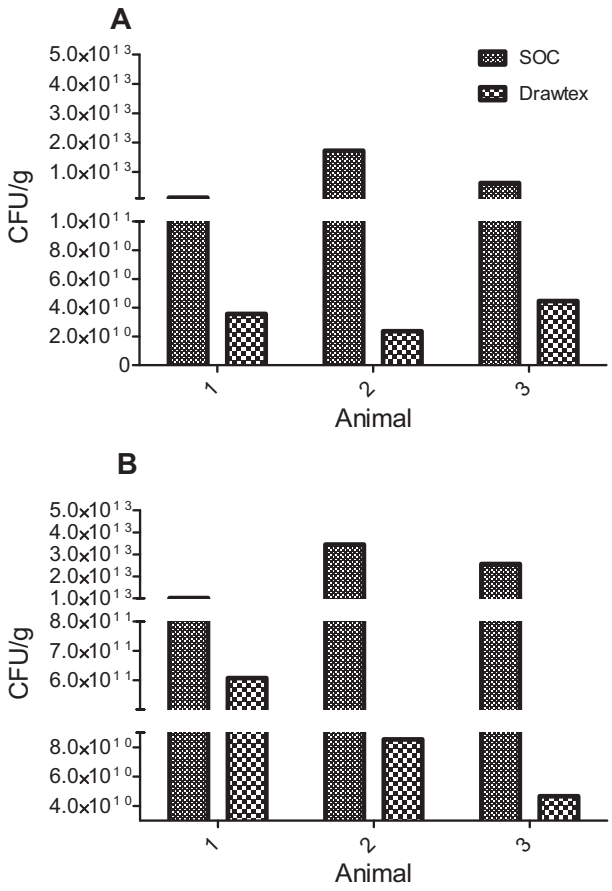

Figure 2. Effects of standard of care and Drawtex on Methycillin-resistant *Staphylococcus aureus* levels in inoculated burn wounds. Data are shown as colony-forming units per gram (CFU/g).

sutured over each of the paired burn wounds. These gauzes were covered by Mepitel One dressing (Mölnlycke, Gothenburg, Sweden).

On post-burn day 2, approximately 24 hours post-inoculation, the rats were anesthetized and dressings and gauzes were removed. Digital images were taken of both wounds, and 2 mm punch biopsies were collected. On each animal, one wound was covered with Mepilex AG (Mölnlycke, Gothenburg, Sweden) representing standard of care (SOC) dressing, and the remaining wound was covered with Drawtex.

On post-burn days 3 and 4, biopsies were collected from both wounds and from the Drawtex dressing material. Digital images were taken on day 4.

Quantitative Cultures

The Drawtex and wound biopsies were weighed and homogenized in sterile saline using a LabGen Homogenizer (Omni International, Kennesaw, GA). The homogenates were then serially diluted and plated on mannitol salt agar plates selective for *Staphylococcus* species. After incubation, yellow colonies (which indicated coagulase positivity and presumptive pathogenic *Staphylococcus* species) were counted and CFU per gram calculated. Data were plotted using GraphPad Prism (GraphPad, La Jolla, CA, Version 5.04).

In Vitro *Protein Absorption*

To examine the protein absorbency of Drawtex, an *in vitro* experiment was conducted. Sterile glass flasks that contained 2 mg/ml, 1 mg/ml, 0.125 mg/ml, or 0.075 mg/ml Bovine Serum Albumin (BSA; Roche USA) in 1X PBS (Phosphate-buffered saline) were set up on a rocker. Pieces of Drawtex, with similar weight and size, were submerged in each of the flasks and allowed to incubate, with constant gentle rocking for 1 hour. One flask containing 2 mg/ml BSA did not contain any Drawtex and served as a control. At 0, 10, 30, 45, and 60 minutes post-submergence of Drawtex, a sample of the media was collected from each of the flasks and BSA concentration was measured using a bicinchoninic acid (BCA) assay (ThermoFisher Scientific, Waltham, MA). Amount of change over time was compared to concentration at time of submergence (t = 0), and data were plotted using GraphPad Prism. This experiment was done in triplicate (n = 3 for each treatment). Significant differences from control (no Drawtex) were assessed using a two-way ANOVA.

In vitro *Bacterial Absorption*

To examine the bacterial absorption properties of Drawtex, two sterile glass flasks containing 50 ml of Todd Hewitt (TH) broth with MRSA (1 x 10^8 CFU per ml) were prepared. A piece of Drawtex was submerged into the media in one of these two bacteria-containing flasks. A third flask contained TH broth only (without inoculum) and also had a piece of Drawtex, equal in weight, submerged in it. The flasks were allowed to sit with gentle rocking for 90 minutes. At 0, 10, 30, 45, and 60 minutes, samples of Drawtex and TH broth from each flask were collected. Quantitative cultures were performed as described above. Amount of change in CFU/g over time was calculated versus CFU/g at t = 1 minute. This experiment was performed in triplicate (n = 3). Significant differences from control (flask with no Drawtex in MRSA culture) were assessed using a two-way ANOVA.

Results

Burn Wound Infection Model

Digital photographs of wounds on post-burn day 4 revealed differences in the clinical appearance between the SOC-treated wounds and the Drawtex-treated wounds (**Figure 1**). Grossly, the SOC wounds showed more evidence of necrosis, while the Drawtex-treated wounds appeared more viable.

No MRSA was detected in any of the baseline biopsies or baseline Drawtex samples. Drawtex-treated wounds had lower bacterial counts on both days 3 and 4 compared to the SOC-treated wounds (**Figure 2**). The lowest bacterial counts (2.37 x 10^{10} CFU/g) were seen in a Drawtex-treated wound on day 3, while the highest bacterial counts (3.44 x 10^{13} CFU/g) were found in an SOC-treated wound on day 4.

In vitro *Protein Absorption*

BSA was measurable in all samples throughout the time course. No significant differences were found between BSA levels in the control compared to both the 0.125 mg/ml and 0.075 mg/ml BSA solutions (**Figure 3**). Beginning 10 minutes after submergence of Drawtex, there was a significant difference in BSA levels in both the 2 mg/ml (0.844) and 1 mg/ml (0.805) solutions. These levels were significantly lower compared to the control. The 2 mg/ml BSA solution had the greatest decrease in BSA level at 1 hour (0.719). Levels of BSA in the control 2 mg/ml (without Drawtex) remained fairly constant throughout the time course (amount of change from t = 0 is 1).

In vitro *Bacterial Absorption*

No MRSA was detected in any of the baseline Drawtex samples (pre-submergence), or in the uninoculated TH broth throughout the time course. No significant differences in MRSA growth existed between the two MRSA cultures at 1 minute after Drawtex submergence; therefore, data were compared to t = 1 minute to determine the amount of change. Starting 10 minutes after Drawtex submergence, the MRSA-containing medium with Drawtex submerged showed a significantly lower bacterial count compared to the control MRSA culture (without Drawtex). This culture had the highest amount of change of bacterial count (2.71) at 90 minutes. Correspondingly, significantly higher bacterial counts were measured in the Drawtex material that was submerged in the culture media, also compared to the control, with the lowest amount of change (0.1590) at 90 minutes ($P < 0.001$, **Figure 4**).

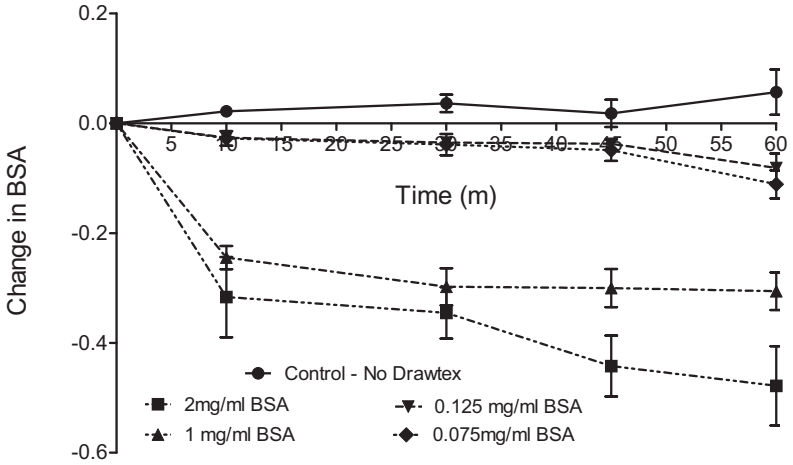

Figure 3. Effects of Drawtex in various BSA levels compared to the control data are shown as amount of change in mg/ml from t = 0. Data points are displayed as the mean (n = 3) ± SD. Statistical significance was determined by two-way ANOVA ($P < 0.001$).

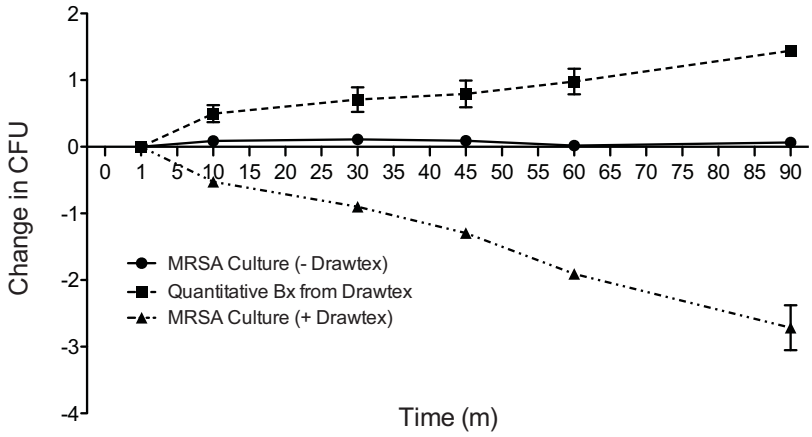

Figure 4. Effects of Drawtex on bacterial counts of MRSA-containing Todd Hewitt Broth. Data are shown as amount of change in colony forming units per gram (CFU/g) from minute 1. Data points are displayed as the mean (n = 3) ± SD. Statistical significance was determined by two-way ANOVA ($P < 0.001$).

Discussion

Though Drawtex has been reported to have exceptional capabilities in absorbing and wicking away exudate and wound debris from wound surfaces, no published *in vitro* studies have been found that quantify these capabilities. The *in vitro* experiments described here were aimed at characterizing the absorption ability of Drawtex at both cellular and molecular levels. These experiments demonstrated a significant reduction in bacterial counts in the MRSA-containing media that had Drawtex submerged in it, while simultaneously showing a significant increase in bacteria in the Drawtex material itself. The logical conclusion is that Drawtex is capable of absorbing bacteria from media to a large extent.

Protein assay data also demonstrated a significant reduction in protein concentration over time in the 2 mg/ml and 1 mg/ml BSA solutions that contained Drawtex, highlighting this property and suggesting that this material would be capable of wicking away other proteins, such as virulence factors, in a wound environment. Further work will be aimed at determining virulence-factor absorption *in vivo*.

This study also utilized a reproducible burn wound infection model that has been developed to allow observation of the effectiveness of wound dressings on local wound infections. Though several clinical case studies have described the use Drawtex to treat a variety of wounds, there have been no controlled pre-clinical studies published comparing Drawtex to a known dressing in burn wounds. Some of these studies have reportedly demonstrated a reduction in both eschar and exudate at the wound areas.[5] In our model, quantitative cultures revealed a reduction of bacterial growth in the Drawtex-treated, MRSA-infected wound area compared to the SOC wound. Further, digital images demonstrated a noticeable difference in viability between the two wounds.

This study demonstrates the ability of Drawtex to reduce bacterial growth in an MRSA-infected burn wound. The *in vitro* work also demonstrates the ability of Drawtex to absorb both protein and bacteria. Additional work is needed to further characterize the mechanisms by which Drawtex impacts wound healing, focusing on its absorptive capabilities. ■

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