

# Laboratory evaluation of Drawtex Hydroconductive Dressing with LevaFiber technology

- **Objective:** The aim of this study was to assess absorption, sequestration and retention of microorganisms by Drawtex Hydroconductive dressing with LevaFiber™ technology.
- **Method:** The absorption over time and the ability to sequester and retain bacteria were assessed in the laboratory using an *in vitro* model where known amounts of fluid and microorganisms were allowed to absorb and sequester over a 24 hour period. The reduction in numbers of microorganisms following sequestration was determined using standard plate counting methods. Retention of the organisms was visualised by scanning electron microscopy.
- **Results:** Drawtex was shown to absorb eight times its own weight in fluid over time and it showed a 90% reduction in bacterial numbers over a 24 hour period in sequestration experiments. Direct observation by scanning electron microscopy demonstrated bacterial retention in the dressing fibres.
- **Conclusion:** Drawtex is a recent addition to the formulary for absorbent dressings available in the UK. It demonstrates excellent absorption properties, while maintaining integrity. In addition, it sequesters and retains microorganisms, which will help with removal of exudate and bioburden from the wound bed to help facilitate wound healing.
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Drawtex; sequestration; electron microscopy; hydroconductive technology

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**W**ound healing is a physiological process which may be impaired in patients with an underlying illness. There are numerous reasons why a wound could become chronic and remain so, including poor vascular supply, poor patient nutrition, and an unhealthy or infected wound bed. The factors causing chronicity of a wound may differ in individual patients but the presentation is similar. The patient often presents with a wound that is producing excess exudate, which is made up of water, proteins, inflammatory mediators, growth factors, different cell types, elevated levels of matrix metalloproteinases (MMPs)<sup>1</sup> and other deleterious enzymes which can cause wound maceration. In addition, there are often high numbers of bacteria<sup>2</sup> which can further hinder the healing process.

The concept of wound bed preparation was described by Falanga et al., in 2000<sup>3</sup>, who provided a framework for assessment and appropriate treatment strategies. The authors suggested that there were clear barriers to healing that needed to be addressed before the progression to recovery could begin. These were described as the presence of devitalised tissue, an increase in the bacterial burden and an imbalance in the moisture at the wound bed. A practical tool frequently used in wound bed preparation is the TIME framework which has been recently reviewed and updated,<sup>4</sup>

and is used within the European Wound Management's Association (EWMA) wound bed preparation document.<sup>5</sup> TIME is used to assess the status of tissue (necrosis, colour, presence of slough etc), infection and inflammation status, the moisture balance and epithelial advancement.

In clinical practice it is relatively easy to observe and evaluate whether a dressing is controlling the level of exudate in a wound during day to day management. However, unless the wound is clinically infected,<sup>6</sup> it is difficult to assess whether there are changes in bacterial burden over time purely by clinical observation. In chronic wounds, it has become widely accepted that wound bioburden usually exists as a biofilm.<sup>7</sup> A biofilm is comprised of a community of microorganisms attached to the wound bed and embedded in a polymeric matrix, shedding free floating bacterial strains to aid with invasion and further colonisation. The microenvironment of a biofilm protects it from the host's innate immune response, where it can live in a symbiotic relationship ad infinitum until this relationship is interrupted.<sup>8</sup>

Debridement can disrupt a biofilm and *in vitro* studies have shown there is a small therapeutic window when topical treatments can be effective.<sup>9</sup> There are numerous methods used to debride devitalised tissue; sharp debridement is still considered the quickest method but is only carried out by experienced practitioners. As many wounds are

managed in primary care, autolytic debridement is preferred using endogenous proteolytic enzymes mediated by specialist dressings and a moist wound environment.<sup>10</sup> The devitalised tissue becomes rehydrated and separates from the viable tissue.<sup>11,12</sup>

An ideal dressing can aid in the debridement of devitalised tissue, absorb exudate and reduce the bacterial bioburden in the wound bed. If all three factors are adequately controlled then the host factors at the wound bed will have opportunity to help the wound heal. Drawtex Hydroconductive Dressing with LevaFiber technology has been available in the UK for almost one year and there is increasing evidence that it can contribute to wound bed preparation by promoting debridement of devitalised and/or necrotic tissue and reducing exudate volume.<sup>13</sup> A recent publication demonstrated that when used according to the manufacturer's guidelines, Drawtex can proactively remove both slough and necrosis rapidly and safely in a wide range of wound aetiologies.<sup>14</sup> This study also demonstrated that the dressing can facilitate moisture balance in the wound bed, as it has the capacity to absorb large volumes of exudate into the dressing, both vertically and horizontally.<sup>14</sup> By layering the dressing, the harmful effects of exudate and its components are removed from the wound and surrounding skin, which may reduce the risk of damage through maceration of delicate tissue. According to the manufacturers, the dressing can be cut to fit any shape or size, which makes the dressing conformable in difficult to dress areas and it can retain its structure even when full of exudate and therefore can be used in cavities and sinuses. While reduction of the necrotic burden and its ability to control excess moisture has been demonstrated,<sup>13</sup> its use when managing bacterial bioburden has not been explored. Therefore, this study was designed to investigate the *in vitro* absorbency and sequestration effects of Drawtex and its suitability as a new dressing for wound bed preparation.

## Method

### Rapid absorbency

The rapid absorbency of six random samples of Drawtex dressing (5x5cm in size) was assessed using two methods: wet weight and residual volume. All dressings were accurately weighed prior to the addition of any fluid using an Adventurer Pro 2 point precision balance. A single 5x5cm square of dressing was placed into a petri dish. Two 10ml volumes of phosphate buffered saline (PBS) were added until the dressing was fully soaked and there was visible residual fluid in the dish. The dressings were left at room temperature to absorb the fluid for 15 minutes in a closed petri dish. Three dressings were proc-

essed using wet weight by removing the dressing from the petri dish, draining residual fluid and reweighing the wet dressing. The absorbency was calculated using the equation:

Fluid Absorbed (g) = wet weight (g) – dry weight (g)

Three dressings were processed for residual volume, where the residual fluid was removed using calibrated micropipettes, and the level of fluid absorbed calculated using the following equation:

Fluid absorbed (ml) = total volume of fluid added (20ml) – residual fluid (ml).

### Absorbency in an *in vitro* model

Six petri dishes were filled with 20ml of PBS and were covered with a layer of aluminium foil with a central hole (2x2cm) cut into it. The dressings (5x5cm) were placed across the opening and secured with masking tape. A glass bottle containing water (total weight=80g/25cm<sup>2</sup>) was placed across the dressing. This allowed the dressing to be in constant contact with the surface of the fluid, resulting in the absorption of fluid over time. This set-up was left for 4 hours and 24 hours at room temperature, where the residual fluid in the petri dish was then accurately measured using calibrated micropipettes to determine the amount of fluid absorbed into the dressing. All tests were carried out in triplicate.

### Sequestration and retention of bacteria using an *in vitro* model

An overnight broth culture of *Escherichia coli* was diluted in sterile PBS to a concentration of 2.2x10<sup>6</sup> cfu/ml, which would maintain organism numbers without allowing growth. Petri dishes were filled with 20ml of the organism suspension. Six petri dishes were covered with a layer of aluminium foil with a central hole (2x2cm) cut into it. The dressings (5x5cm) were placed across the opening and secured with masking tape. A glass bottle containing water (total weight=80g/25cm<sup>2</sup>) was placed across the dressing as a weight, allowing the dressing to touch the surface of the fluid. These were left at room temperature for 4 and 24 hours. After each respective time period, the set-up was taken down and the residual fluid was processed for viable bacterial numbers to determine sequestration and retention. Viable bacterial numbers were determined per ml (cfu/ml) using surface viable counts (Wasp Automated Spiral Plater [Don Whitley Scientific, Shipley, UK]). The tests were carried out in triplicate for both time periods. This was repeated with all organisms. Controls (n=3) were processed in the same manner without dressings for comparison of organism numbers. These experiments were repeated in triplicate for both time points with controls using MRSA at an inoculum of 1.6x10<sup>6</sup>cfu/ml and *Candida albicans* of 1.4x10<sup>5</sup>cfu/ml. Results are presented as mean and standard deviation (SD).

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**Electron microscopy for structural analysis (dry, hydrated and retaining microorganisms)**

The structure of Drawtex dressings was investigated in both hydrated and dry conditions and in the presence of three different microorganisms, *Escherichia coli*, methicillin resistant-*Staphylococcus aureus* (MRSA) and *Candida albicans*, using a Zeiss 40VP scanning electron microscope. A low acceleration voltage of 1keV and below 100pA electron beam current allowed balance of surface charges and image samples without additional conductive surface coatings.

**Preparation of the dressings for scanning electron microscopy.**

- Dry dressings: The dressings were trimmed to approximately 0.5cmx0.5cm and attached to ubiquitous electron microscopy pin stubs. The dressing was scanned and images taken at varying magnification to investigate the fibre surface, morphology and retained bacteria.
- Hydrated dressings: Sterile distilled water was added to a 1.0cmx1.0cm piece of dressing until completely hydrated. Excess water was clearly visible. The dressings were left to absorb the water for a minimum of 2 hours and then prepared for electron microscopy. The system for processing the hydrated samples was manufactured and installed by Quorum Technologies. The dressing was trimmed to 0.5x0.5cm and placed into a mechanical holder. This holder was dipped into a liquid nitrogen slush bath to freeze the dressing in its fully hydrated state at approximately -210°C. The holder with the sample was then transported under partial vacuum in a specimen transfer device and attached to the scanning electron microscope cryo-preparation chamber. The vacuum in the preparation chamber was created by a rotary pump. Partial water sublimation was performed at -120°C (time taken was between 1–3 hours). The sample was then transferred into the scanning electron microscopy chamber onto the liquid nitrogen vapour cooled stage. The images were taken at the same magnification of the dry dressings to allow measurement of the hydrated fibres compared to dry fibres. (Note: the radius of the dressing fibres were measured, not the length).
- Dressing with microorganisms: A suspension of microorganism in PBS prepared as described in the

sequestration *in vitro* model experiment was sequestered into the dressing for 24 hours. The dressing was then vortex-mixed in PBS to help remove bacteria not attached to the dressing and then washed a further three times in PBS. The dressing was then placed into formal saline for 18 hours to kill the microorganism (to ensure the microscope will not be contaminated). The dressings were then washed three more times with PBS to remove traces of formaldehyde and any further residual microorganism not firmly adhered to the dressings. The dressings were then placed at 37°C for 24 hours to dry the dressings, ready for viewing by scanning electron microscopy. The dressing was trimmed to 0.5cmx0.5cm and attached to the mount prior to insertion into the electron microscope. Any change in structure of dressing was noted (in case of change following sequestration) and the position of microorganism on the dressing noted. The differences between groups were determined using a two-tailed t test; p<0.05 was considered significant.

**Results**

**Rapid absorbency**

The rapid absorbency (15 minutes) of individual samples of Drawtex undertaken using residual volume and wet weight were compared. Absorbency was calculated as 7.87(SD 0.43)ml using residual volume and 7.89(SD 0.01)ml using wet weight. Residual volume was used to calculate absorbency in the model system at 4 hours and 24 hours. The Drawtex dressing absorbed up to five times its own weight in fluid after 15 minutes and 4 hours. When left for a 24 hour period, this increased to over eight times its own weight. The absorbency values calculated by residual volume are shown in Table 1. The dressing absorbed different amounts of fluid in the model system over time indicating that the fluid continued to be absorbed over a 24 hour period at room temperature. Drawtex is a flexible and soft dressing when dry. When saturated with fluid, it retains its physical structure and does not release any fibres or particles into the surrounding fluid.

**Sequestration and retention of bacteria**

The sequestration and retention of microorganisms into the dressing varied depending upon the microorganism and the time period. At 4 hours, there was a reduction in the numbers of organisms held in a suspension of 4.5%, 82.1% and 99%, respectively, of *Escherichia coli*, MRSA and *Candida albicans*. This increased to 29%, 97.6% and 99.7% respectively at 24 hours, indicating that Drawtex sequestered the microorganisms over time and retained them within the structure of the dressing. There was increased numbers of MRSA and *Candida albicans* sequestered and retained, compared to *Escherichia coli*.

**Table 1. Shows the weight of the dressing and the amount of fluid absorbed into the dressing in 15mins, and 4 hours and 24 hours in a model system using residual volume.**

Weight(g) (SD) at baseline	Absorbency After 15mins (ml)(SD)	Absorbed in model (ml)(SD) after 4hrs	Absorbed in model (ml)(SD) after 24hrs
1.61 (0.05)	7.87 (0.43)	8.34 (0.1)	14.03(0.38)

### Electron microscopy

- Dry dressings: When observed in a dry state, Drawtex™ consisted of a random mesh of fibres, all of a similar size. There were three variations of the fibres: smooth, fine striated, and large-striated. The mean diameter of the fibres were smooth, 16.5µm; fine-striated, 21.5µm; and large-striated, 25µm. The appearance of the fibres are shown in Fig 1 at 1000x magnification.

- Hydrated dressings: the hydrated state of the dressings closely resembled the dry state. The dressings consisted of a mesh of fibres where the striations were still apparent, but less marked. There was apparent adherence of an unknown substance to the fibres following hydration, but this may just have been an artefact of the hydration process. The dimensions of the fibres appeared to be swollen compared to the dry dressing and the mean diameters of the individual fibres were increased to smooth (22.5µm), fine-striated (25µm) and large-striated (27.5µm). Following hydration, the diameters of the fibres all increased in size, but the diameter of the smooth fibres increased the most from 16.5µm to 22.5µm (27% increase). This is shown in Fig 2 at 1000x magnification.

### Retention of bacteria

Figs 3–6 demonstrate the sequestration and retention properties of the dressing. All microorganisms were easily observed attached to the dressing and could be seen in every field. This demonstrated that the microorganisms were sequestered into the dressing and remained attached to the dressing following very vigorous washing.

### Discussion

Drawtex is a modern hydroconductive dressing that is used to prepare the wound bed by debriding slough and necrotic tissue. It has also been recognised as an excellent absorber of excess exudate, contributing to the reduction of bioburden, toxins such as MMPs and devitalised, sloughy tissue. It is uncommon for a dressing to both debride and absorb simultaneously, giving this dressing dual functionality. Therefore, this study was undertaken to evaluate the absorbency and the ability for the dressing to sequester and retain microorganisms using an *in vitro* model system.

Initially, two methods were compared to determine rapid absorbency of the dressing, the first using wet weight of the dressing and the second using residual fluid volume. The methods were reproducible and comparable, with the method using wet weight of the dressing showing fluid absorbed at 7.89ml compared to 7.87ml using residual fluid volume method with no significant difference observed ( $p=0.808$ ; paired t-test). Determining the wet weight of the dressing is generally consid-

Fig 1. Scanning electron micrograph of Drawtex at 1000x magnification.

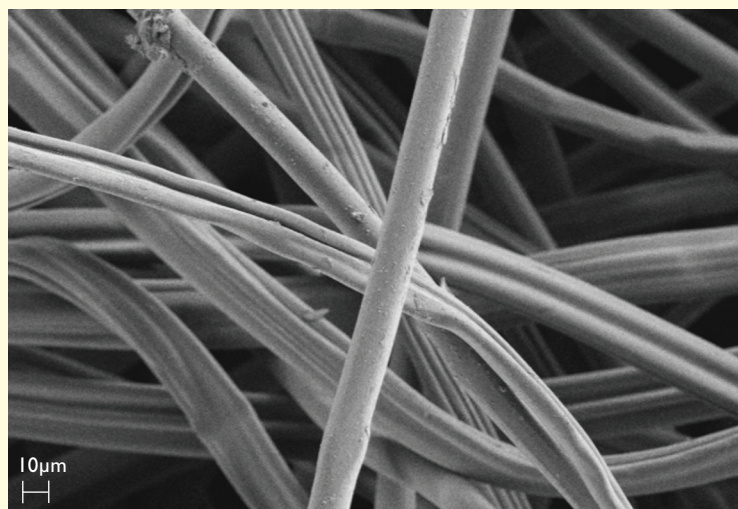


Fig 2. Drawtex hydrated under SEM at 1000x magnification



ered a more accurate method of assessing fluid absorption, while residual volume is considered to be inherently more inaccurate. In this study, the use of calibrated automatic pipettes helped reduce inaccuracies; results were not significantly different. In addition, in later experiments for absorbency and sequestration with the *in vitro* set up, using wet weight to determine absorbency may have created other inaccuracies, because moisture evaporating from the dressing over the 24 hour period could not be controlled for in the experimental design. For these reasons, residual volume was chosen as the preferred method for measuring absorbency in this study; it is important to be aware of its limitations.

A 5x5cm piece of Drawtex dressing with an average weight of 1.61g was shown to rapidly absorb over 5 times its weight (7.87ml) in just 15 minutes. When

Fig 3. Drawtex with *E.coli* attached to the fibres (7.27K magnification).

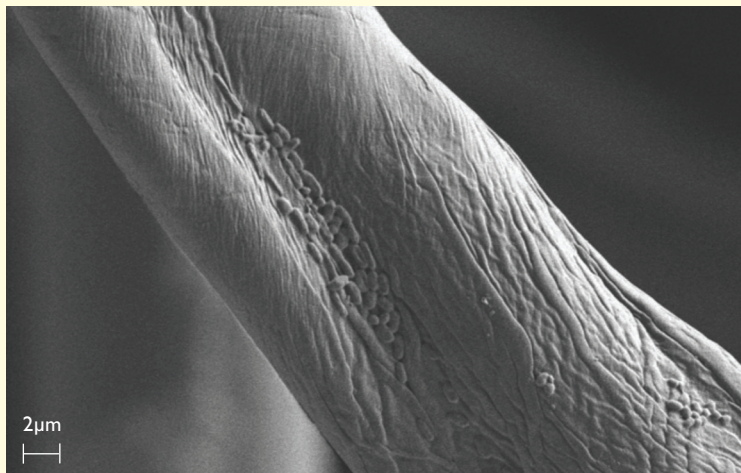


Fig 4. Drawtex with attached MRSA (14.14K magnification).

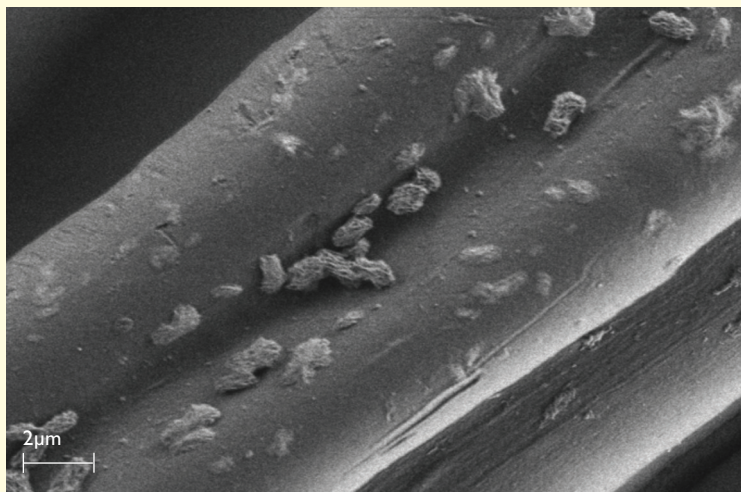
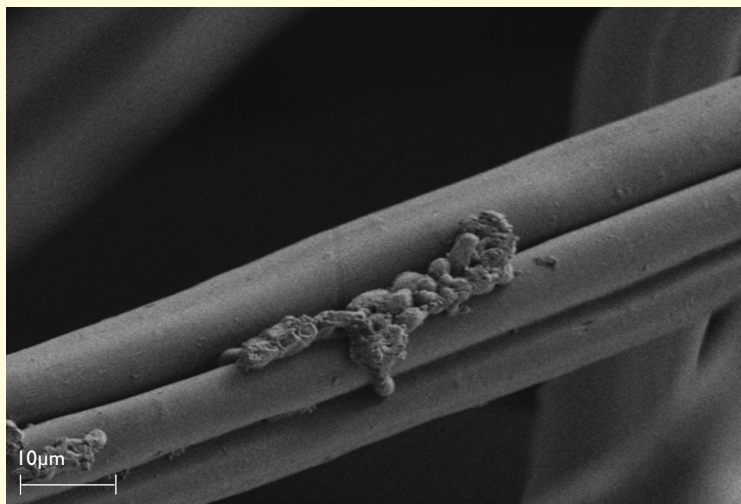


Fig 5. Drawtex with *Candida albicans* attached (3.84K magnification).



the dressing was placed in continuous contact with the surface of the fluid under a similar pressure as seen under compression, continuous absorbency was observed. At 24 hours, the fluid absorbed into the dressing had increased up to eight times its own weight, with 14.03ml of fluid being absorbed through the dressing of 25cm<sup>2</sup> in a 24 hour period at room temperature. Although this model system only determined the fluid absorbed by measuring residual volume and did not take moisture vapour transmission into account during this experimental procedure, and increase in absorbency could still be clearly demonstrated. In addition, when the dressing was fully saturated, it retained its structure and did not release any fibres into the surrounding fluid.

Drawtex sequestered microorganisms from the fluid showing a percentage reduction of 4.5–99% in organism numbers within a 4 hour period, which increased to 25–99.7% within 24 hours. This was dependent upon the organism tested. All organisms have the ability to move within the fluid either through direct movement due to the presence of flagella on the surface of the organism (*Escherichia coli*) or by Brownian motion (MRSA and *Candida albicans*). Furthermore, if the dressing showed a strong wicking action, it would be expected that organisms would be drawn into the dressing during this process. The ability of the dressing to retain the microorganisms depended upon the chemistry of the fabric and the ensuing interaction of the microorganism and the dressing. Microorganisms have a negative charge on their surface and it is possible that the dressing attracted the bacteria through an electrostatic attraction or alternatively, Van der Waal forces or hydrophobic-hydrophilic reaction. In this study, the Gram positive bacterium MRSA and the yeast *Candida albicans* exhibited a higher rate of retention by the dressing than the Gram negative bacterium *Escherichia coli*. There are known structural differences in the cell envelope of all the three microorganisms tested and these may have accounted for the differences in retention observed. In a clinical situation, the retention of microorganisms in a dressing is important because bioburden can be reduced, allowing the wound to heal. To further elucidate how the dressing was able to retain the organisms, further *in vitro* studies could be carried out to determine the strength of binding of the cells and the exact mechanism of attachment. In addition, the physical nature of the material of the dressing would have to be analysed further to determine this effect.

The fibres of the dressing were visualised using scanning electron microscopy. Under dry conditions, fibres appeared slightly different structurally, described in this study as smooth, fine-striated and large-striated. Following hydration, the diameters of the fibres all increased in size but the diameter of the smooth fibres increased the most from 16.5µm

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to 22.5µm (approximately 27%). Whether it is possible to increase fluid absorption capabilities by modifying the ratio of the smooth to striated fibres is worthy of further investigation, but was outside the scope of this study.

Adherence of microorganisms to the dressing was seen using scanning electron microscopy. The microorganism were firmly attached to the surface of the fibres and were also attached to each other, seen by the linear formation of the Gram negative bacillus, *E. coli* and the clustering appearance with *S. aureus* and *C. albicans*. In most photographs taken by scanning electron microscopy, the retained organisms were observed on the striated fibres in preference to the smooth fibres. Whether this was due to the difference in the physical nature of the material or just due to the striations could not be determined in these studies; further research on the structure of individual fibres and their impact on function is required.

### Conclusion

In this study, Drawtex demonstrated excellent *in vitro* absorption, sequestration and retention of fluid and microorganisms without loss of integrity of the dressing itself. The evidence to date suggests that it can provide additional benefits to other passive wound care products because it can be layered on very heavily exuding wounds, allowing the excess exudate to be removed from the wound interface. It can also be cut to any shape and does not shed fibres or fall apart, even when soaked. While many antimicrobial dressings are produced to reduce the microbial bioburden, it is also important to evaluate the effect of non-antimicrobial dressings in the management of microbial bioburden at the wound interface. Further study investigating the effect on formation or reduction of biofilm (either by reduction in fluid or nutrient availability) would be an essential next step in full *in vitro* evaluation of this new product. ■

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