J. of Wound Care, 23(3) (2014) 118-27

Evaluation of Drawtex™, i*n vitro* and *In vivo* effects

Valerie Edwards-Jones[1] Vladimir Vishnyakov[2] and Pam Spruce [3]

[1] School of Research, Enterprise and Innovation, Manchester Metropolitan University, Manchester, UK.

[2] Current address: School of Computing and Engineering, The University of Huddersfield, Queensgate, Huddersfield H1 3DH, UK

Contact details: ++44161 247 1432

Email v.e.jones@mmu.ac.uk

[2]School of Engineering, Manchester Metropolitan University, UK

[3]TVRE Consulting. Stoke on Trent, UK

Key words:

Drawtex, sequestration, electron microscopy, hydroconductive technology

Abstract

Good wound bed preparation is an essential aspect of wound care and effective wound healing. Removal of dead and necrotic tissue either through autolytic or interventional debridement, followed by good exudate management, inhibition of matrix metalloproteases and bacterial bioburden control should allow the chronic wound to process to closure. It is known, still, that wound healing in these circumstances is not a simple process and that maintaining a healthy wound bed is central to the process.

Many practitioners rely on the TIME (Tissue, Infection/Inflammation, Moisture balance and wound Edge) framework to help them with wound bed preparation and there are a variety of dressings available to help with debridement, exudate management, reduction of bacterial bioburden and inhibit metalloproteases. The sequence of application of the various dressings will depend upon their function. This study describes the function of a dressing, Drawtex, a hydroconductive dressing, which can be used to assist with wound bed preparation through its absorption, sequestration and retention properties. The absorption over time, ability to sequester and retain bacteria were assessed in the laboratory using a variety of methods. 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 24hr period in sequestration experiments. Utilisation of direct observation by scanning electron microscopy demonstrated bacterial retention in the fibres.

Introduction

Any wound should heal unless the patient has some underlying condition that prevents it. There are a number of reasons why a wound will remain in a chronic condition including poor vascular supply, poor patient nutrition, and an unhealthy 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 contains water, proteins, inflammatory mediators, growth factors, different cell types and elevated levels of matrix metalloproteases (MMPs)[1] and other deleterious enzymes which can cause wound maceration. In addition, there are often high numbers of bacteria[2] which can cause further problems to the healing process.

A practical tool frequently used in wound bed preparation is the TIME framework which has been recently reviewed and updated[3] and is used within the European Wound Management's Association (EWMA) wound bed preparation document[4]. TIME is used to assess the status of tissue (necrosis, colour, presence of slough etc), infection and inflammation status, the moisture balance and the epithelial advancement.

Removing necrotic tissue is an essential part of wound management as this enables full assessment of the tissue and can visualise potential infection. There are numerous methods used to debride devitalised tissue and 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[5]. The devitalised tissue becomes rehydrated and separates from the viable tissue[6,7].

Drawtex®, a hydroconductive wound dressing has been available in the UK for almost one year and there is increasing evidence that can contribute to wound bed preparation by aiding debridement of necrotic tissue and reduce exudate volume through its LevaFiber™ technology[8]. The dressing can absorb large volumes of exudate into the dressing (volume?), both vertically and horizontally and can be cut to fit any shape or size. It is reported to retain its structure even when full of exudate, on removal from a wound (Drawtex, data on file).

This study was designed to investigate the *in vitro* and *in vivo* absorbency and sequestration effects of Drawtex and its suitability as a new dressing for wound bed preparation.

Materials and Methods

Dressing used: Drawtex ™ (Beier Drawtex) (5 x 5cm) Batch number A00010 2015-02

Microorganisms used: *Escherichia coli* NCTC 9001 , *Staphylococcus aureus* (MRSA type 16), *Candida albicans* MDH Serotype A

Experiment 1 Absorbency

The absorbency of the dressing was assessed by placing a 5X5cm square of each dressing into a Petri dish. 10ml volumes of Phosphate Buffered Saline (PBS) was added until there was visible residual fluid in the dish. The dressings were left at room temperature to absorb the fluid for 15minutes and then the residual fluid was removed and the level of fluid absorbed calculated.

Experiment 2 Absorbency in a model system.

Six Petri dishes were filled with 20ml of PBS and the Petri dishes were covered with a layer of aluminium foil with a central hole (2 x2cm) cut into it. The dressings (5x5cm) were placed across the opening and secured with masking tape. A universal glass bottle containing water (total weight =approx 80g, 60mm Hg) was placed across the dressing as a weight and this allowed the dressing to continually touch the surface of the fluid, resulting in the fluid being allowed to absorb over time. The model was left for 4hrs and 24hrs at room temperature. The model was taken down and the residual fluid in the Petri dish accurately measured to determine the amount absorbed into the dressing.

Experiment 3. Sequestration and retention of bacteria using a model system.

An overnight broth culture of *E.coli* was diluted in sterile PBS to a final concentration of approximately 10^6 cfu/ml. This would retain the levels of bacteria without allowing growth. Petri dishes were filled with 20ml of the suspension. Six Petri dishes were covered with a layer of aluminium foil with a central hole (2 x2cm) cut into it. The dressings (5x5cm) were placed across the opening and secured with masking tape. A universal glass bottle containing water (total weight =approx 80g, 60mm Hg) was placed across the dressing as a weight and this allowed the dressing to touch the surface of the fluid. These were left at room temperature for 4 and 24hrs. At the respective time period, the model was taken down and the residual fluid processed for viable bacterial numbers to determine sequestration and retention. This was repeated with all organisms. Controls were processed in the same manner without dressings for comparison of organism numbers.

Experiment 4. Assessment of Biomass following sequestration at 24hrs.

Following completion of the sequestration studies the dressings were removed from the model, washed with PBS following vortex mixing to remove the bacteria not retained on the dressing and placed in the 37^oC degree incubator till fully dried. The dressings were then accurately weighed to determine the amount of biomass (reflecting the weight of microorganisms) retained on the dressings. Dressings containing only PBS were also processed in the same manner to ensure accuracy of biomass and to ensure increase/decrease occurred due to mass of bacteria rather than chemical changes to the dressings.

Experiment 5: Electron Microscopy of dressings (Dry, full hydrated and retaining microorganisms)

The structure of Drawtex™ dressings were investigated in fully hydrated and dry conditions and in the presence of three different microorganisms, *Escherichia coli,* methicillin resistant *Staphylococcus aureus* and *Candida albicans* using a Zeiss 40VP scanning electron microscope. Low acceleration voltage of 1 keV and below 100 pA electron beam current allowed to balance 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 0.5cm x 0.5cm and attached to the ubiquitous electron microscopy pin stubs. The dressing was scanned and images taken at varying magnification to investigate the fibre surface morphology and retained bacteria.

Fully Hydrated dressings.

Sterile distilled water was added to a 1cm x 1cm piece of dressing until fully hydrated. Excess water was clearly visible. The dressings were left to absorb the water for a minimum of 2 hrs and then prepared for the SEM. The system for processing the hydrated samples was manufactured and installed by Quorum Technologies. The dressing was trimmed to0.5 x0.5cm and placed into a mechanical holder. This holder was dipped into liquid nitrogen slush bath to freeze the dressing in a fully hydrated nature at approximately 63 K (-- 210^{o} C) at constant vacuum pumping. The holder with the sample was then transported under partial vacuum in specimen transfer device into attached to the SEM cryo-preparation chamber. The vacuum in the preparation chamber was created by a rotary pump. Partial water sublimation was done at -120^{o}C (time taken between 1-3hrs). . The sample then was

transferred into the SEM 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:

Three microorganisms were used to observe retention. *E.coli, Pseudomonas aeruginosa, Candida albicans,* and methicillin resistant *Staphylococcus aureus* (MRSA)

A suspension of microorganism in PBS was allowed to sequester into the dressing overnight. The dressing was 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 18hrs to kill the microorganism (to ensure the microscope was not contaminated). The dressings were then washed three times with PBS to remove traces of formaldehyde and also to remove any further residual microorganism not firmly adhered to the dressings. The dressings were then placed at 37^{o}C for 24hrs to dry the dressings ready for scanning electron microscopy.

The dressing was trimmed to 0.5cm x 0.5cm 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.

Results;

Absorbency:

Drawtex™ absorbed up to five times its own weight in fluid after adding it to the dressing at fifteen minutes and four hours. If left for a 24hr period this increased to over eight times its own weight. The absorbency values are shown in table 1. Drawtex™ was flexible and soft dressing when dry and it retained its physical structure and did not release any fibres or particles into the surrounding fluid. The dressing absorbed different amounts of fluid in the model system over time indicating that the fluid absorbed was evapourating over a twenty four hour period at room temperature.

Table 1 shows the amount of fluid absorbed into the dressing in 15mins, 4 hrours and 24hours.

Weight(g) (SD)	**Fluid added (ml)**	**Residual fluid (ml)(SD)**	**Absorbency After 15mins (ml)(SD)**	**Absorbed in model (ml)**(SD) **after 4hrs**	**Absorbed in model (ml)**(SD) **after 24hrs**
1.61(0.05)	10	2.13(0.40)	7.87 (0.43)	8.34 (0.1)	14.03(0.38)

Sequestration and retention of bacteria

The sequestration and retention of microorganisms into the dressing varied depending upon microorganism and the time period. At 4hrs there was a reduction in the numbers of organisms held in suspension of 0.02 log (4.5%), 0.8 log (82.1%) and and 2.1 log (99%) respectively for *E.coli, S,aureus* and *C.albicans.* This increased to 0.12 log (25%), 1.6 log (97.6%) and 2.57 log (99.7%) respectively at 24hrs. Drawtex™ sequestered the

microorganisms over time and retained them within the structure of the dressing. There was more *S. aureus* and *Candida albicans* sequestered and retained, compared to *E.coli.*

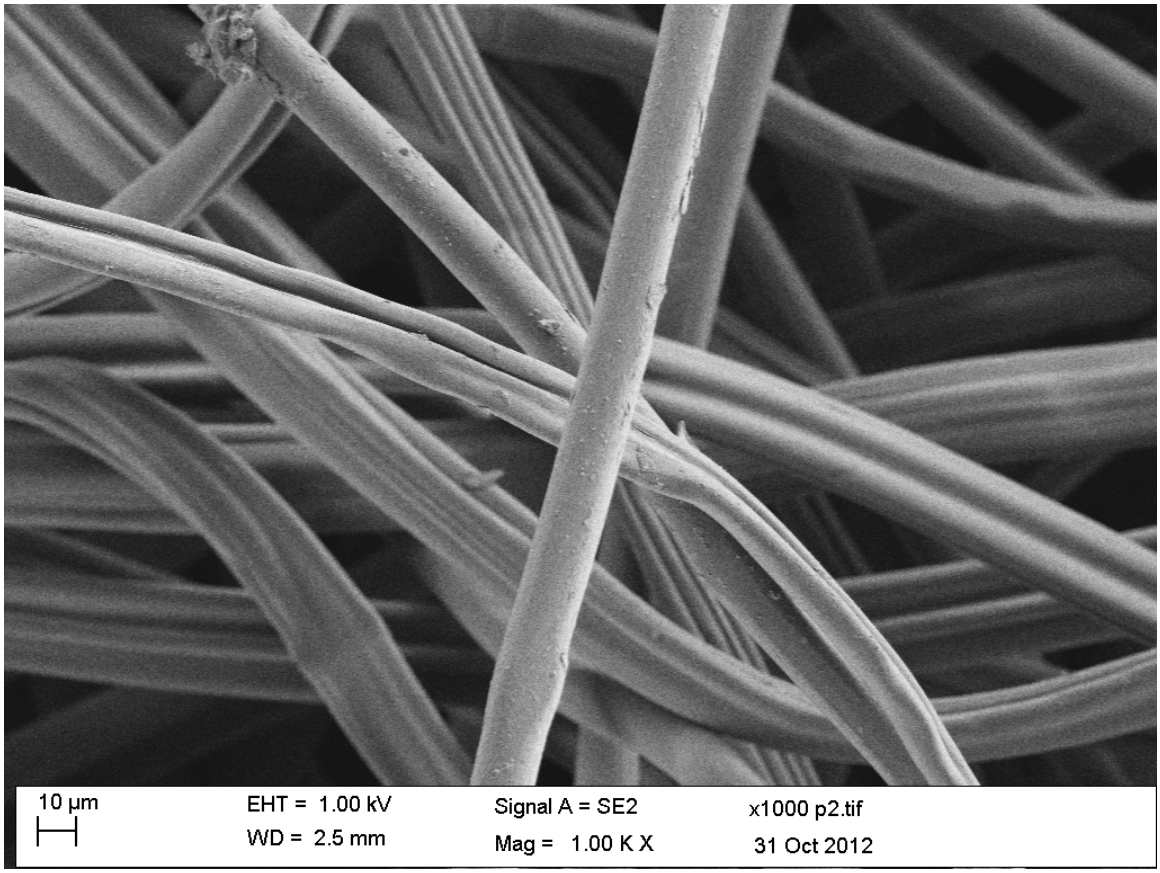
Following removal from the model system the dressings were washed thoroughly with water and then dried and re-weighed to determine if there was any biomass (equating to the microorganism) retained in the dressing. The biomass was determined as 0.1g of MRSA and 0.08g of *E.coli* at 24hrs. There was a 0.06g biomass of *C. albicans* determined at 24hrs, however, this could not be directly compared to the bacterial biomass as there were lower numbers of fungal cells in the model system from the onset.

Electron Microscopy

Dry

When observed in a dry state, Drawtex ^{TM} consisted of a random mesh of fibres, all of a similar size**.** There were three variations of the fibres,: smooth, fine striated, large striated**.** The mean diameter of the fibres were , smooth 16.5µm, fine striated 21.5 µm and large striated 25 µm .The appearance of the fibres are shown in figure 1 at 1000 times magnification.

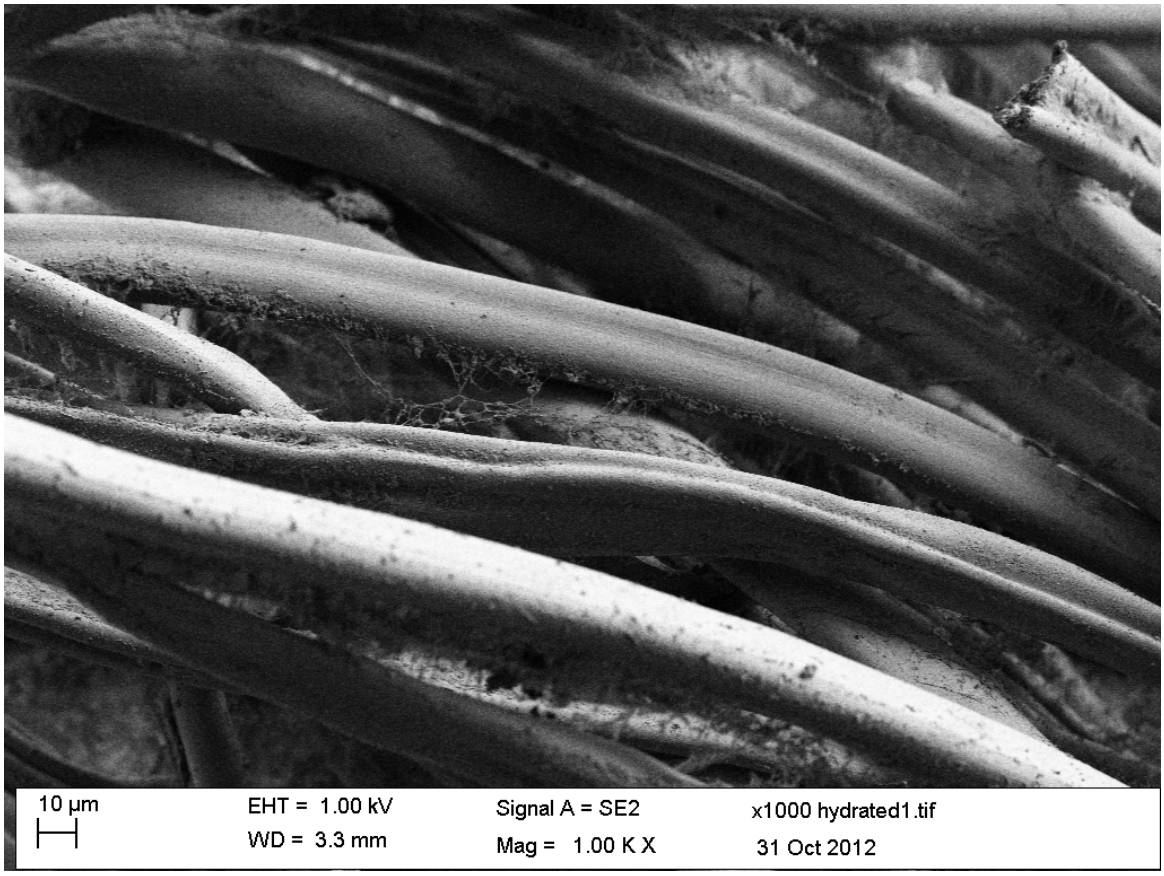
Figure 1 Scanning electron micrograph of Drawtex^{TM} at 1000 times magnification.



Hydrated

The dressing looked very similar hydrated and consisted of a mesh of fibres, the striations were still apparent but less marked. There was some residual adherence of 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 22.5 µm, fine striated 25 µm and large striated 27.5 µm . This is shown in figure 2 at 1000 times magnification.

Figure 2 Drawtex™ hydrated under SEM (1000 times magnification)



Retention of bacteria

A series of figures (figure 3-6) are shown to 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.

The microscope allowed variable magnification and the most appropriate for photography were used for image capture.

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

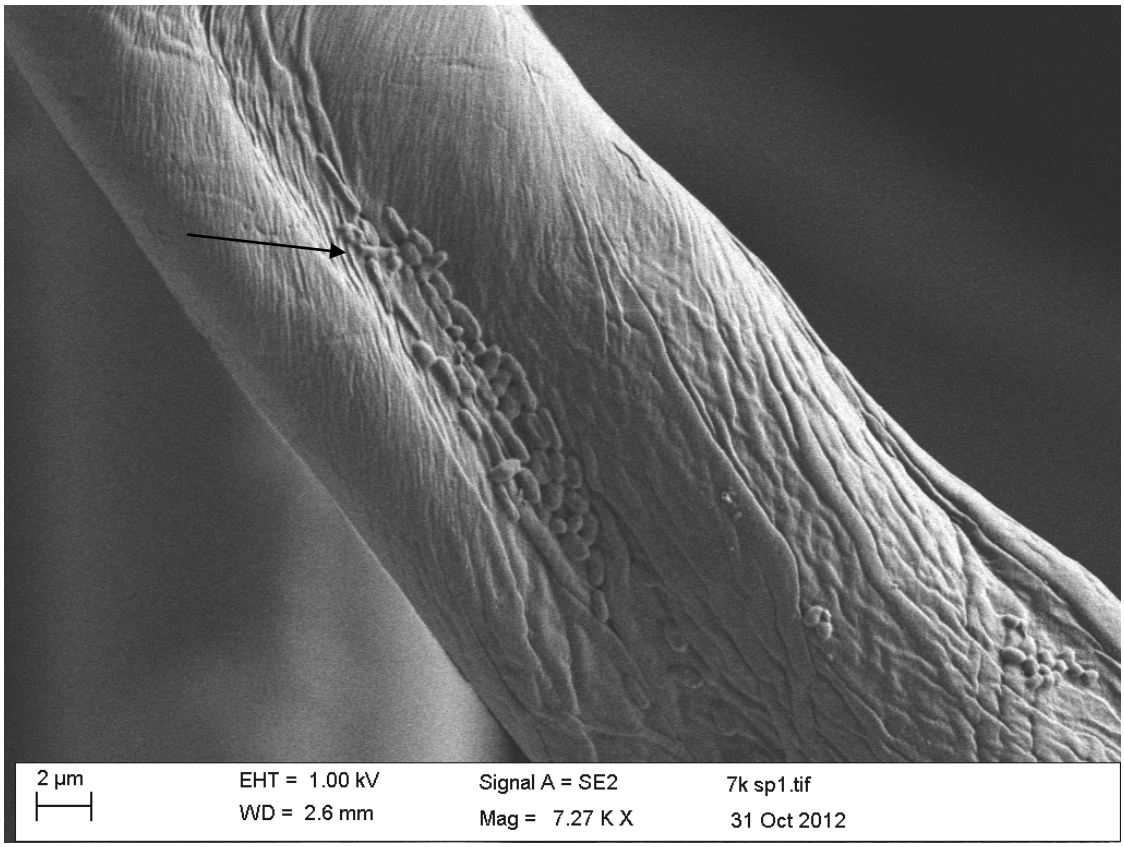


Figure 4 Drawtex™ with MRSA attached to the fibres (14.14K magnification).

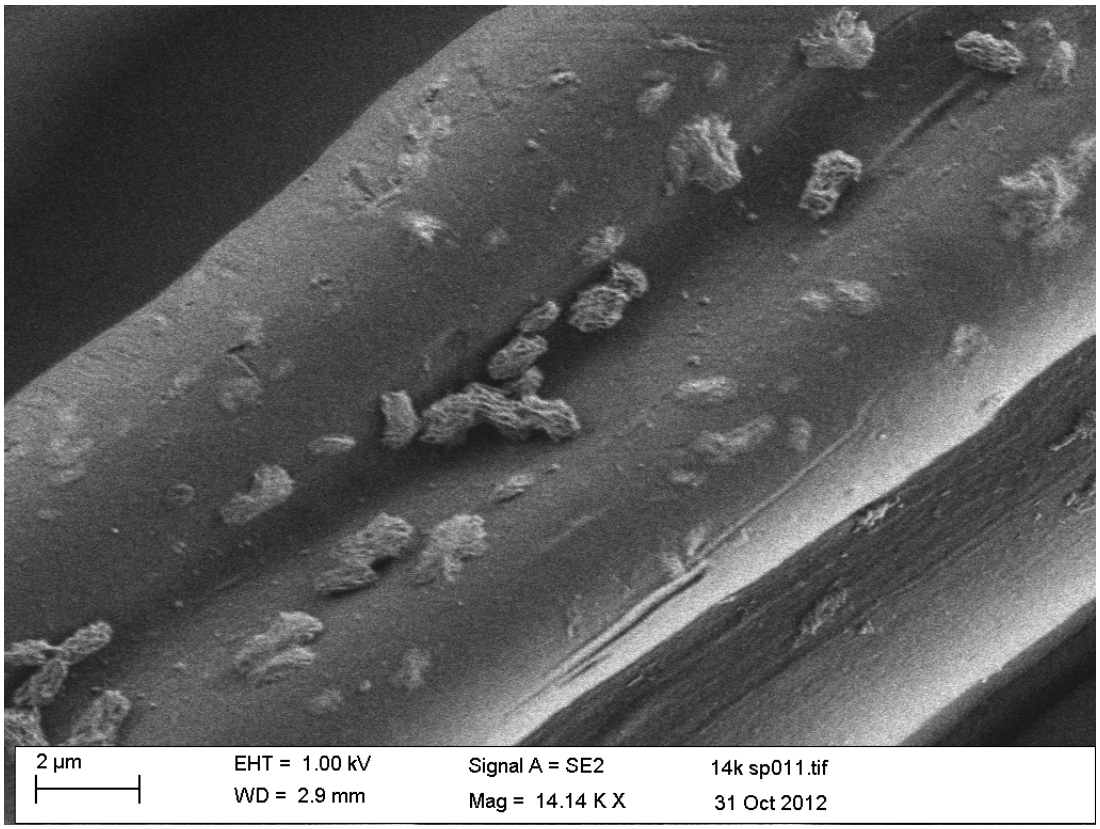
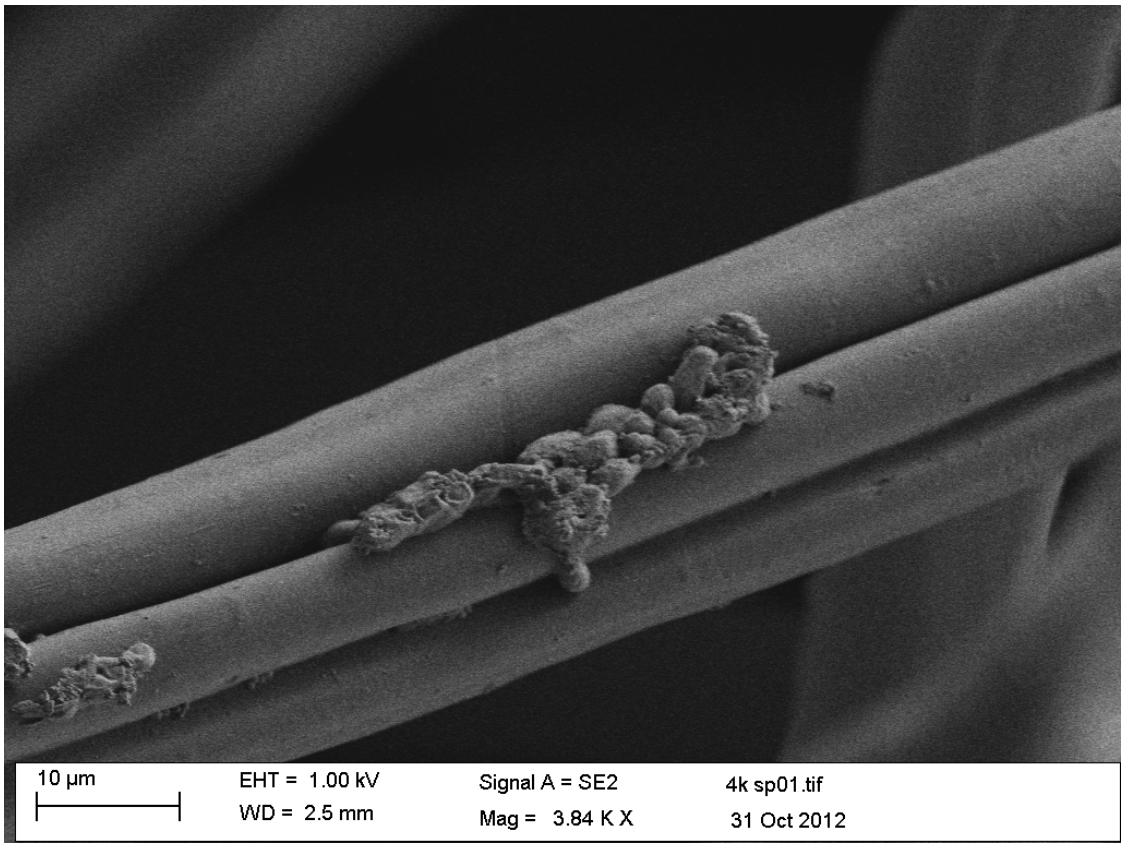


Figure 5 Drawtex™ with *Candida albicans* attached to the fibres (3.84K magnification).



Discussion

Drawtex™ is a modern hydroconductive dressing that is used in wound bed preparation, particularly with debridement. It is also an excellent at absorption of excess exudate, reducing bioburden, toxins such as MMPs and devitalised, sloughy tissue. It is unusual for a dressing to both debride and absorb simultaneously and this gives this dressing a dual purpose. The LevaFiber™ technology of the dressing allows it to lift, hold and transfer exudate both vertically and horizontally into the body of the dressing.

This study was undertaken to evaluate the absorbency and the ability for the dressing to sequester and retain microorganisms *in vitro* and to assess its effects *in vivo* . Initially the ability to absorb fluid was undertaken and it was shown that a 5 x 5cm dressing with an average weight of 1.61g could absorb over 5 times its weight in just 15 minutes. When placed in a model system where the dressing was also to continuously be in contact with the dressing under a similar pressure as seen under compression at room temperature, there was continuous absorbency observed. At 24hrs the fluid transferred into the dressing had increased up to eight times its own weight. This may be due to the dressing having a good moisture vapour transmission rate with 14ml of fluid being absorbed through the dressing of 25cm^2 in a 24hr period at room temperature (24 ^{o}C). When the dressing was fully saturated it retained its structure and did not release any fibres into the surrounding fluid which is essential in wound care.

Drawtex™ sequestered microorganisms from the fluid showing a percentage reduction of 4.5-99% in organism numbers within a four hour period which increased to 25-99.7% within 24hrs. This was dependent upon the organism tested. All organisms have the ability to move within the fluid either through direct movement because of flagella present on the surface of the organism (*E.coli*) or by Brownian motion (MRSA and *C.albicans)* and 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 would depend 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 a n electrostatic attraction or alternatively it may be another type of molecular force such as Van der Waal forces or hydrophobic-hydrophilic reaction. 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 observed using scanning electron microscopy and under dry conditions there were three different fibres observed with slightly different microscopic structures describes as smooth, fine striated and coarse striated. Following hydration the diameters of the fibres all increased in size but the diameter of the smooth fibres increased the most from 16.5 µm to 22.5 µm (approximately 27%).

Retention of the microorganisms to the dressing was seen using scanning electron microscopy. All microorganisms were easily detected being observed in nearly every field viewed. 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 bacilli (*E.coli and P.aeruginosa*) and the clustering appearance with *S.aureus* and *C.albicans*.

Drawtex™ did demonstrate excellent *in vitro* absorption, sequestration and retention of fluid and microorganisms without loss of integrity of the dressing itself. This was also demonstrated in the two case studies where it demonstrated effective wound bed preparation which makes it a possible alternative to passive absorptive products, like calcium alginates, hydrofibers, foams and superabsorbers. Drawtex™ can provide additional benefits both to wound care specialists and patients because it can be layered on

the very heavily exudating wounds, it can be cut and tailored to any shape or does not shed fibres or fall apart, even when soaked.

References

1. Chen WYJ, Rogers AA, Lydon MJ. (1992) Characterization of biologic properties of wound fluid collected during early stages of wound healing. *J Investigative Dermatol* 99(5): 559–64.

2. Bowler,PG., Duerden, BI., Armstrong, DG (2001) Wound microbiology and associated approaches to wound management *Clin Micro Reviews* ; 14, 2, 244-269.

3. Leaper DJ, Schultz G, Carville K. (2012) Extending the TIME concept: what have we learned in the past 10 years? *Int Wound J.* 9 (Suppl. 2) 1–19

4. European Wound Management Association (EWMA) (2004) Position Document: *Wound Bed Preparation in Practice*. London: MEP Ltd

5. Strohal R, Apelqvist J, Dissemond J, et al. (2013) EWMA Document: Debridement. *J Wound Care*. 22 (Suppl. 1): S1–S52

6. T (2011) Wound debridement in the community setting. Br J Community Nurs 16(6 Suppl): 14–20

7. Acton C, Chadwick P, et al. (2011) Consensus guidance for the use of debridement techniques in the UK. *Wounds UK.* 7; 1: 77–84

8. Ochs D, Uberti G, Donate GA, et al. (2012) Evaluation of mechanisms of action of a hydroconductive wound dressing (Drawtex) in chronic wounds. *Wounds* September 2012 Supplement