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Effectiveness of a monofilament wound debridement pad at removing biofilm and slough: ex vivo and clinical performance

***Gregory S. Schultz**,[1] PhD; **Kevin Woo**,[2] PhD RN FAPWCA Associate Professor; **Dot Weir**,[3] RN, CWON, CWS; **Qingping Yang**,[4] MS

***Corresponding author email:** schultzg@ufl.edu

1 Department of Obstetrics & Gynecology, University of Florida, Gainesville, Florida, US. **2** School of Nursing, School of Rehabilitation Therapy, Faculty of Health Sciences, Queen's University, Kingston, Ontario, Canada. **3** Catholic Health Advanced Wound Healing Centers, Buffalo, NY, US. **2** The Institute for Wound Research at the University of Florida, Department of Obstetrics & Gynecology, University of Florida, Gainesville, FL, US.

Effectiveness of a monofilament wound debridement pad at removing biofilm and slough: *ex vivo* and clinical performance

Objective: Removal of slough and other devitalised tissue is an important step in biofilm-based wound care (BBWC) and wound bed preparation. Debridement is key to management of both slough and biofilm, and a number of methods are available to achieve this, including surgical/sharp and mechanical debridement. Developments have led to products indicated for debridement of wounds, including a sterile pad consisting of monofilament fibres. Our aim is to examine the effectiveness of a monofilament wound debridement pad (WDP), Debrisoft.

Method: We assessed the WDP, in laboratory tests, for the removal of mature biofilm from porcine dermal tissue in an *ex vivo* model, and the clinical management of sloughy wounds that would benefit from debridement. We used the UPPER score to determine the superficial infection status.

Results: The WDP was effective in removing biofilm from porcine dermal tissue. A case series of 10 patients with chronic wounds suggested that the WDP was beneficial in the removal of slough. All chronic wounds had slough and were cleaned weekly, for four weeks, using the MDP to achieve improved healing and a clean wound bed. The average wound size decreased from 8.09cm^2 at baseline to 2.3cm^2 at week four, with three wounds healed completely. Exudate was reduced, and the UPPER score improved in every patient.

Conclusion: These results indicate that the WDP effectively debrides biofilm and slough, and contributes to care that follows the principles of wound bed preparation and BBWC.

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biofilm • chronic wound • debridement • monofilament pad • slough • surgical wound

Biofilm is associated with multiple diseases in humans that are characterised by chronic infection and inflammation,[1] and it is recognised as an impediment to the healing of chronic wounds, such as venous leg ulcers (VLU), pressure ulcers (PU) and diabetic foot ulcers (DFU).[2] Biofilm structures were initially reported to be detected in over 60% of chronic skin wounds,[3,4] while a recent meta-analysis indicates the actual prevalence in chronic skin wounds is closer to 80%.[5] The variation in the reported proportion of wounds affected is likely to be because of the methods used to isolate and identify biofilm, rather than the true presence or absence. Biofilm is a natural phenotype for microorganisms in most environments, and it is found in, essentially, all environments in which it is has been sought.[6] As a complex ecosystem, biofilm protects the organisms in it, reducing the effectiveness of antimicrobial agents and the natural defences against infection.[7,8]

Investigations, in both animal models of impaired healing and in clinical studies, support a direct role for biofilm in impairing healing and contributing to the chronicity of skin wounds.[9] Chronic wounds are classified and clinically treated, based on their primary aetiology: VLU due to impaired venous function, PU caused by pressure and shear damage, and DFU resulting from a combination of peripheral neuropathy, trauma, and peripheral arterial disease. Despite these different classifications, the biological and molecular mechanisms that underpin tissue breakdown and recalcitrance in chronic wounds are similar.[10,11] Chronic skin wounds, typically, are 'stuck' in a state of chronic inflammation that leads to chronically elevated levels of proteases (matrix metalloproteinases, MMPs, neutrophil elastase) and reactive oxygen species (ROS). Prolonged and excessive inflammatory responses lead to degradation of proteins essential for healing, including growth factors, their receptors and extracellular matrix proteins (ECM).[10] Since biofilm has been shown to stimulate chronic infection and inflammation in 18 other human pathologies,[1] interest is now centred on the role that biofilm plays in chronic inflammation and infection in human chronic skin wounds.[12]

The principles of wound bed preparation (WBP) that were introduced in 2003[13] have been enlarged to include the concepts of biofilm-based wound care (BBWC),[14] which emphasise the need to effectively remove slough and devitalised tissues from a wound and, equally importantly, to also remove biofilm that is often associated with slough and devitalised tissue.[15,16]

***Gregory S. Schultz**,[1] PhD; **Kevin Woo**,[2] PhD RN FAPWCA Associate Professor; **Dot Weir**,[3] RN, CWON, CWS; **Qingping Yang**,[4] MS
***Corresponding author email:** schultzg@ufl.edu
1 Department of Obstetrics & Gynecology, University of Florida, Gainesville, Florida, US. **2** School of Nursing, School of Rehabilitation Therapy, Faculty of Health Sciences, Queen's University, Kingston, Ontario, Canada. **3** Catholic Health Advanced Wound Healing Centers, Buffalo, NY, US. **2** The Institute for Wound Research at the University of Florida, Department of Obstetrics & Gynecology, University of Florida, Gainesville, FL, US.

BBWC also emphasises combining the effective debridement of biofilm with the application of topical antimicrobial agents that can kill remaining planktonic bacteria, and prevent reformation of biofilm, which can occur within three days.[12,17] In addition, the recently published Consensus Guidelines for the Identification and Treatment of Biofilms in Chronic Non-Healing Wounds emphasises that standard of care for chronic skin ulcers must include treatment measures to remove/disrupt biofilm and suppress regrowth.[18] Several products for debriding loose, sloughy material from a wound are available, including a monofilament wound debridement pad, Debrisoft (Lohmann & Rauscher, Germany). The monofilament wound debridement pad (WDP) is a soft, fleecy pad containing millions of polyester fibres, each of a special length and density, and cut at an angle to actively loosen debris such as slough, necrosis and hyperkeratosis, rapidly and safely, binding it to the fibres within the pad. Laboratory studies have demonstrated the effectiveness of the debridement pad in the removal of biofilm from surfaces.[19] Laboratory studies are instructive in understanding the function of a wound management product and its feasibility for clinical use. Nevertheless, the direct relevance of such studies to the effectiveness of products in the human clinical situation is limited.[11,20] While the human wound is the optimal model for studying healing, it is not always possible due to ethical and safety concerns. Explanted skin models offer an alternative that closely resemble the clinical situation and emulate the full complexity of a human chronic wound.[11,20]

Here we describe the effectiveness of the WDP in the removal of *Pseudomonas aeruginosa* biofilm in an *ex vivo* porcine skin explant model,[21] and a series of 10 clinical cases that illustrate the effect on chronic wounds in which slough requiring debridement was identified.

Table 1. Baseline demographics, wound type, duration, and wound management products used in the 10-patient clinical case series

Patient ID	Age	Sex	Wound aetiology	Wound duration	Comorbidities	Other wound treatment
1	45	M	DFU	2 weeks	Diabetes, hypertension	AQUACEL Ag+, Mesorb, Tegaderm
2	57	F	Trauma	1 week	Hypertension, osteoporosis	Hydrogel, Mesorb, Tegaderm
3	66	M	Venous	23 days	Angina, IHD	Hydrofera blue, Coban 2
4	75	F	Venous	2 weeks	Venous disease	Hydrofera blue, Coban 2
5	78	F	Pressure	4 weeks	Diabetes, PAD, MVG	AQUACEL Ag+, Mesorb, Tegaderm
6	82	F	Pressure	4 weeks	Spinal cord injury, recurrent UTI	Alginate, Mesorb, Tegaderm
7	55	M	Surgical	10 days	Diabetes, rentinopathy, renal insufficiency, PAD	Betadine gauze dressing
8	62	F	Surgical	3 weeks	Diabetes, osteoporosis, reflux disease, hypertension, fatty liver	Betadine gauze dressing
9	74	M	Surgical	15 days	Diabetes, hypertension, stroke, heart failure, renal failure, PAD	Betadine gauze dressing
10	78	M	Pressure	3 weeks	Seizure, spinal chord injury	Iodosorb, Mepilex foam dressing, 3 weeks, thereafter, Mesorb, Tegaderm

PAD—Peripheral arterial disease; UTI—Urinary tract infection; IHD—Ischaemic heart disease; MVG—mitral valve regurgitation; DFU—diabetic foot ulcer

Materials and methods

Ex vivo porcine skin biofilm model

Sterile porcine skin explants with partial-thickness wounds were prepared according to published methods.[2,21] Briefly, sheets of fresh porcine skin (~30cm x 30cm) obtained from an USDA-approved commercial meat processing facility were thoroughly cleaned, closely trimmed, and the subcutaneous fat layer trimmed away leaving a skin sample approximately 1–2mm thick. A single, partial thickness excision wound (50mm x 100mm, 0.8mm deep) was created in the centre of the dorsal surface of each explant using a Paget electric dermatome. Explants were sterilised by submersion in phosphate buffered saline (PBS), containing 0.6% hypochlorous acid and 0.5% Tween, followed by exposure to a chlorine gas chamber and submersion in PBS, containing 0.6% hypochlorous acid and 0.5% Tween 80, for five minutes. After rinsing twice in sterile PBS, explants were transferred into 245mm x 245mm x 2mm (500cm^2) sterile bioassay dishes (Nunc 240835) containing sterile 0.5% soft tryptic soy agar with antimycotic (amphotericin B 2.5µg/ml) and antibiotic (gentamicin 50µg/ml) to limit biofilm growth to the explants and inhibit fungal growth.

Mature *Pseudomonas aeruginosa* biofilms were created on each sterile explant. Planktonic culture (100µl) containing approximately 10^7–10^8 colony forming units per millilitre (CFU/ml) of early log phase growth (0.2–0.6OD 640nm) planktonic *Pseudomonas aeruginosa*, strain PAO1 were inoculated onto the wound area. Inoculated explants were incubated (three days, 37°C, 5% CO_2) in air saturated with water vapour. Explants were transferred daily to fresh sterile 0.5% soft TSA supplemented with antibiotic and antimycotic.

Debridement was conducted by a laboratory technician (QY) and a clinician (DW), in a laminar flow hood, according to the manufacturer's instructions. A WDP (4 x 4cm) was removed from the packaging and hydrated with 20ml of sterile saline. Explants were secured to large pieces of sterile cardboard with large sterile paper clamps and placed on the sterile surface of the laminar flow hood. A hydrated WDP was held in one hand, pressed firmly to the surface of the explant and moved laterally across the surface of the explant, approximately four times, using motion and pressure typical for clinical debridement of a patient's wound

bed. A second new WDP was hydrated and the debridement process was repeated. Sterile gauze pads (8-ply 4'x 4'; 10cm x 10cm, Kendall) were placed in a sterile 150mm Petri dish and moistened with 20ml, or 40–60ml of sterile saline, respectively, dispensed directly onto the pads. Excess saline was gently squeezed out immediately before use. WDP and gauze pads were used, without folding. Debridement was conducted by holding the pad in one hand and wiping back and forth, and in a circular motion using gentle pressure appropriate to normal clinical use.

CFUs of total *Pseudomonas aeruginosa* bacteria and biofilm *Pseudomonas aeruginosa* bacteria were quantified in six replicate 8mm biopsies taken using disposable skin punches from the inoculated wound area of explants before and after debridement. Total bacterial levels were measured by ultrasonically dispersing the planktonic and biofilm bacteria in biopsies that were transferred into tubes containing 5ml of PBS (containing 5ppm of Tween 20 nonionic surfactant) then sonicated five times in a water bath (at room temperature) for 1.5 minutes, with a one minute pause between each of the sonication cycles. CFUs of *Pseudomonas aeruginosa* biofilm bacteria were measured in 8mm biopsies following submergence in 200µg/ml gentamicin (50 x MIC) for 24 hours to kill all planktonic bacteria, but does not kill bacteria protected in the mature biofilm community. The resulting bacterial suspension was serially diluted into PBS and plated in triplicate onto tryptic soy agar plates (TSA: Becton Dickson Co.). Plates were incubated for 24 hours at 37ºC in a humidified incubator. The colony counts were determined by manual counting, the CFU/ml calculated, and mean counts calculated for each test condition to quantify CFU for total and biofilm-associated bacteria. Statistical assessment of differences was conducted by Kruskal-Wallis ANOVA of log-transformed data. Differences were considered statistically significant if $p<0.05$.

Additional full-thickness biopsies were taken using an 8mm skin punch, fixed with glutaraldehyde (24 hours), and processed for scanning electron microscopy (SEM) by serial dehydration in alcohol solutions, drying in a fume hood and sputter coating in an argon gas atmosphere with gold and palladium.[21] Specimens were imaged using a Hitachi S-4000 SEM.

Fig 1. Mean colony forming units (CFU) of total planktonic and biofilm-associated *Pseudomonas aeruginosa* before and after debridement with WDP or gauze conducted by laboratory technician (QY)

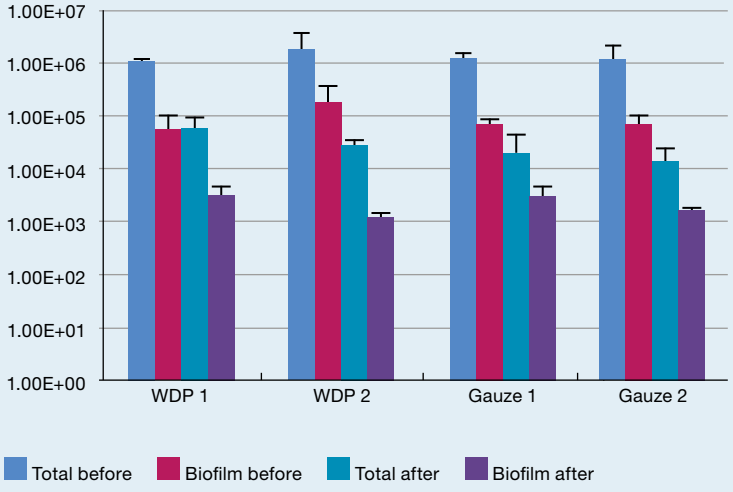
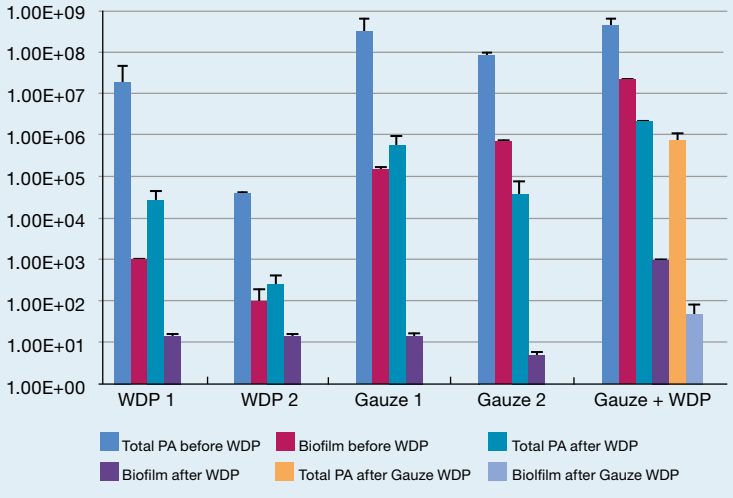


Fig 2. Mean colony forming units (CFU) of total planktonic and biofilm-associated *Pseudomonas aeruginosa* (PA) before and after debridement with WDP alone or following debridement with gauze, and gauze alone conducted by DW (WDP 1, Gauze 1 and Gauze+WDP) or a QY (WDP 2, Gauze 2)



Clinical case series

Patients with acute or chronic wounds attending the wound management clinic in Canada were managed in a product evaluation plan using best practice that included WDP. At the start and at week four, the following parameters were assessed and measured for all wounds: size (cm) using a ruler to measure the two largest perpendicular dimensions; percentage of the wound area covered by slough assessed by visual estimation; local wound infection using the UPPER checklist which determines the presence of unhealthy tissue, poor healing, pain, increased moderate-to-heavy exudate and reek ('smell') which are related to local wound infection;[22,23] pain during the debridement procedure (procedural pain) using a verbal analogue scale (VAS) on which '0' was no pain and '10' was the worst pain imaginable.

Wounds that required debridement of slough were debrided with WDP used according to the manufacturer's instructions. WDP was moistened before use with 30–50ml of normal saline (Baxter, Canada). A single WDP was used per patient per week. Wounds were managed using primary and secondary dressings appropriate to the condition and requirements of the wound (Table 1). Once slough was removed and wounds were healing, debridement with WDP was discontinued and standard care continued. None of the patients developed systemic infection or received antibiotics.

Results

Ex vivo porcine skin biofilm model

We prepared eight explants, four to establish pre-debridement baseline counts, two of these were used to establish baseline total *Pseudomonas aeruginosa* counts and two to establish baseline biofilm-associated *Pseudomonas aeruginosa* counts. The other four explants were used to establish post-debridement counts, two for total *Pseudomonas aeruginosa* counts and two for biofilm-associated *Pseudomonas aeruginosa* counts. Explants consistently contained a mean total CFU (planktonic and biofilm-associated *Pseudomonas aeruginosa*) of approximately $1x10^6$ and $1.8x10^6$ *Pseudomonas aeruginosa*, and between $5.3x10^4$ and $1.75x10^5$ biofilm-associated *Pseudomonas aeruginosa* per biopsy (Fig 1). Debridement of two explants, each with WDP or gauze, conducted by a laboratory technician (QY), generated consistent mean post-debridement CFU values for total *Pseudomonas aeruginosa* approximately 1.5 logs lower than those for untreated explants. The count of biofilm-associated *Pseudomonas aeruginosa* also reduced by up to ~2 logs after debridement with WDP. Debridement using gauze resulted in a reduction in total CFU of ~2 logs and of ~1.5 logs for biofilm-associated *Pseudomonas aeruginosa*. These reductions are similar to those observed following debridement using WDP ($p>0.05$).

Separate explant samples were debrided using WDP or gauze by an experienced clinician (DW; WDP 1 and gauze 1) and QY (WDP 2 and gauze 2). DW additionally used a combination of gauze followed by WDP. The findings are presented in Fig 2. In total, 10 explants were used. In explants before debridement (n=2), the counts of biofilm-associated *Pseudomonas aeruginosa* were significantly lower than the total *Pseudomonas aeruginosa* count indicating that a proportion of the *Pseudomonas aeruginosa* were planktonic phenotype. In all explants debrided with WDP (n=2) or gauze alone (n=2), both total and biofilm-associated *Pseudomonas aeruginosa* counts reduced, indicating that WDP effectively removed biofilm. Similar outcomes were observed when debridement was conducted by either DW or QY. In the explants in which gauze and WDP were used sequentially (n=1), only minor additional reductions in both total and biofilm *Pseudomonas aeruginosa* counts were seen indicating that gauze and WDP are equally effective. Subjective observations made when WDP was used for debridement by DW suggest that less pressure was required to remove biofilm than was required when using gauze, suggesting that WDP may be as efficient as gauze but with less likelihood of trauma and pain for patients.

SEM revealed clear differences between the *ex vivo* explants with biofilm before debridement and those debrided with WDP. Biofilm is clearly visible on untreated explants with dense populations of *Pseudomonas aeruginosa* embedded in extracellular polymeric substance (EPS) and the ECM of the dermis masked (Fig 3). Following debridement with WDP the fibrous structure of the dermal ECM is visible with no visible evidence of remaining biofilm exopolysaccharide or organisms (Fig 4).

Fig 3. Scanning electron micrographs (SEM) of biofilm on the surface of porcine *ex vivo* dermal explants before debridement with monofilament wound debridement pad, showing the appearance of biofilm structures at different locations on the explants before debridement with the monofilament wound debridement pad. Note the classical appearance of biofilm exopolymeric matrix with embedded bacterial (*Pseudomonas aeruginosa* rods). Panels (**a**) and (**c**) are at twice the magnification of panels (**b**) and (**d**)

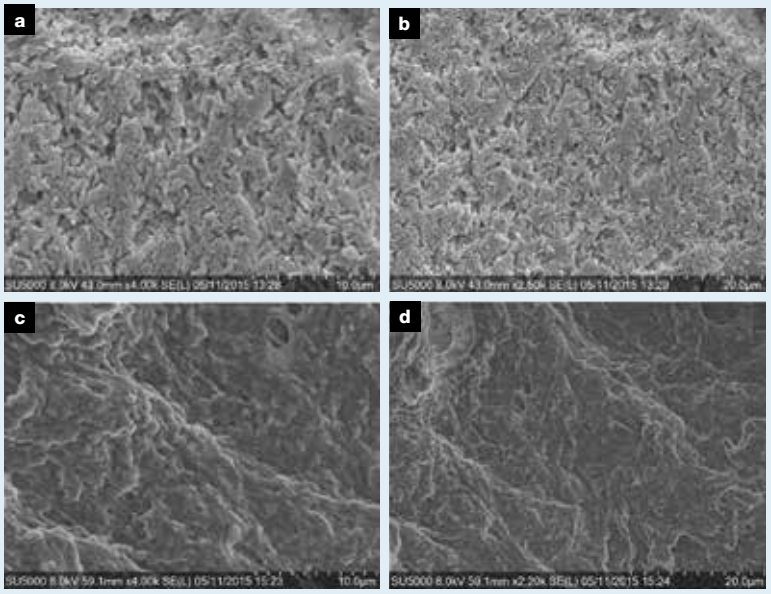
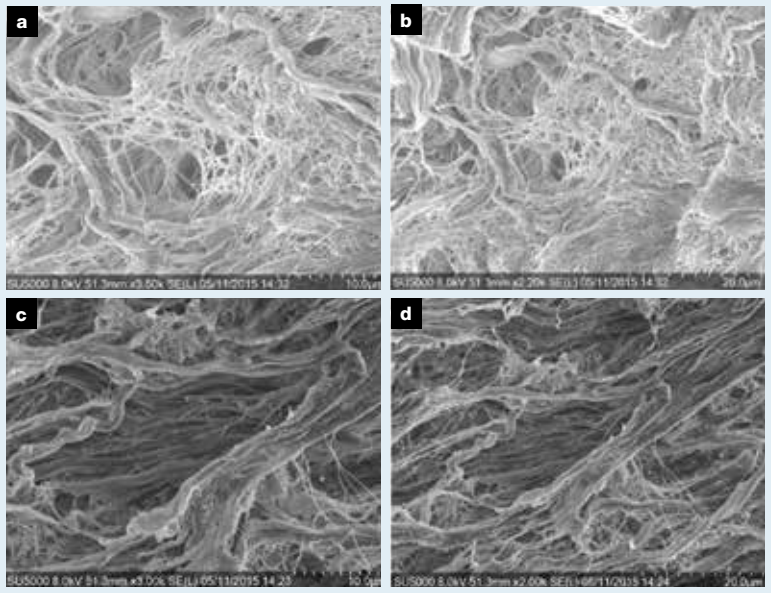


Fig 4. Scanning electron micrographs (SEM) of the surface of porcine *ex vivo* dermal after debridement with monofilament wound debridement pad. The panels show the appearance of different locations on the explants after debridement with the monofilament wound debridement pad. Note the absence of extensive biofilm structures, but the abundant collagen fibres of the extracellular matrix. Panels (**a**) and (**c**) are at twice the magnification of panels (**b**) and (**d**)



Clinical case series

Demographic details for the patients, wound aetiology

Table 2. Outcomes assessed after four weeks' treatment with monofilament wound debridement pad integrated in standard care

Patient ID	Size (cm) at start	Size (cm) at end (% change)	Slough (%) at start	Slough (%) at end	UPPER assessment and score	UPPER score at end	Exudate	Pain score at start and end
1	1 x 1.1	1 x 0.5 (–54.5%)	100	20	Unhealthy tissue, smell, exudate, **score: 4**	0	Reduced	5–5
2	15.2 x 13	13.3 x 11.2 (–24.6%)	90	10	Unhealthy tissue, poor healing, exudate, **score: 4**	1	Reduced	4–5
3	10 x 7.6	1.5 x 1 (–87.5%)	100	0	Unhealthy tissue, smell, exudate, poor healing, **score: 4**	0	Reduced	4–4
4	3.4 x 3.2	2.2 x 3 (–39.3%)	80	0	Unhealthy tissue, pain, smell, exudate **score: 5**	3	Reduced	0–0
5	1 x 0.5	0 (–100.0%)	60	0	Unhealthy tissue, exudate, **score: 3**	0	Reduced	5–5
6	3.5 x 2.3	3 x 2 (–25.5)	70	30	Unhealthy tissue, smell, exudate, **score: 4**	2	Reduced	2–3
7	5.1 x 2.3	2.8 x 0.8 (–80.9%)	60	20	Unhealthy tissue, smell, exudate, pain, poor healing, **score: 5**	1	Reduced	0–0
8	2.1 x 1.2	0 (–100.0%)	100	0	Unhealthy tissue, smell; exudate, **score: 3**	0	Reduced	4–4
9	3.2 x 1.1	0 (–100.0%)	60	0	Unhealthy tissue, poor healing, exudate, **score: 3**	0	Reduced	6–7
10	5 x 3	2 x 1 (–86.7%)	70	0	Unhealthy tissue, exudate, **score: 3**	1	Reduced	4–4

and duration, and other treatments used in the standard of care are shown in Table 1. The wounds treated were surgical (n=3), PU (n=3), VLU (n=2), DFU (n=1), trauma wound (n=1). Patient 4 presented with a complex large-area VLU, and so a small isolated area of the wound was selected for weekly management with WDP. The outcomes for the 10 patients in the case series are shown in Table 2. All wound surface areas reduced over time from mean surface area of 8.09cm^2 at baseline to 2.3cm^2 at week four. In three wounds complete closure was achieved. In a further three, the wound surface area reduced by ≥80%, and in the remaining four, wound surface area reduced by between ~24% and ~55%. At baseline, all wounds were covered with between 60–100% surface area slough (mean: 80%). The surface area of wounds covered by slough reduced in all wounds. In six wounds, slough was removed completely, and five of these achieved a clean granulating surface. In the remaining four, the area of wound covered by slough reduced significantly from a clinical perspective.

The UPPER score for the evaluation of signs and symptoms associated with local wound infection reduced in all patients. In five patients, the score was reduced to 0/5, and in the remaining five the score reduced to 3/5 in two patients and 1/5 in three patients. The amount of exudate reduced in every wound. Procedural pain scores remained similar at the start and end of the evaluation.

Cases

We selected the following four cases to illustrate the effect of WDP on slough in representative wounds.

Patient 3 (Fig 5), a male patient (66 years old) with angina and ischaemic heart disease presented with an active VLU (CEAP Classification 6; 10x7.6cm (79cm^2)) of 23 days' duration on the medial aspect of the right lower leg. The peripheral skin was macerated and the skin around the ulcer was hyperkeratotic, and haemosiderin discolouration was evident. The UPPER assessment score was 4: unhealthy tissue, poor healing, exudate and smell. The ulcer surface was entirely covered by slough leading to the decision to use WDP to debride. Following debridement once a week with WDP, the ulcer was managed with Hydrofera Blue antibacterial foam primary dressing (Hollister Woundcare, US) and Coban 2 compression bandage (3M), changed once weekly. Slough was formally assessed at week zero and week four. It was noted, informally, that slough,0 having been removed by WDP at weekly dressing changes, recurred between treatment episodes. The ulcer surface area covered by slough reduced during treatment from 100% to 0%, the ulcer surface area reduced 98% to 1.5x1cm (1.5cm^2), and exudate had reduced at week four. The UPPER score reduced to 0 at week four. Procedural pain was assessed by the patient to be at level 4 at both week zero and week four indicating that there had been no change in perceived pain levels during management with WDP.

Patient 5 (Fig 6), was a 78-year-old female with diabetes, peripheral arterial disease, and mitral valve regurgitation, presented with a sacral category III PU of four weeks' duration (1x0.5cm (0.5cm^2)). The UPPER assessment score was 3: unhealthy tissue, poor healing

and exudate, and 60% of the ulcer surface was covered by slough leading to the decision to use WDP to debride. Following debridement once a week with WDP, the ulcer was managed with AQUACEL Ag+ (ConvaTec) primary dressing and Mesorb absorbent secondary dressing (Mölnlycke) retained with Tegaderm tape (3M), changed once weekly, and the patient was managed on a low air loss pressure-relieving mattress (Hill-Rom). Slough was formally assessed at week zero and week four. The ulcer surface area covered by slough reduced during treatment from 60% to 0%, the ulcer was completely healed, and exudate had reduced at week four. The UPPER score reduced to 0 at week four. Procedural pain was assessed by the patient to be at level 5 at both week zero and week four, indicating that there had been no change in perceived pain levels during management with WDP.

Patient 6 (Fig 7), a 82-year-old female with a spinal cord injury and recurrent urinary tract infection presented with a sacral category III PU of four weeks' duration (3.5x2.3cm (8.05cm^2)). The UPPER assessment score was 4: unhealthy tissue, poor healing, exudate and smell, and 70% of the ulcer surface was covered by slough leading to the decision to use WDP to debride. One WDP was used per week to remove as much slough as possible. Following debridement, the ulcer was managed with Alginate (Kaltostat, ConvaTec) primary dressing and Mesorb absorbent secondary dressing (Mölnlycke) retained with Tegaderm tape (3M), changed once weekly, and the patient was managed on low air loss pressure-relieving mattress (Hill-Rom). Slough was formally assessed at week zero and week four. The ulcer surface area covered by slough reduced during treatment from 70% to 30%, the ulcer surface area reduced by 25.5% to 3x2cm (6cm^2), and exudate had reduced at week four. The UPPER score reduced to 2 at week four. Procedural pain was assessed by the patient to be at level 2 at week zero and level 3 at week four, indicating that there had been a small increase in perceived pain levels during management with WDP.

Patient 7 (Fig 8), a 55-year-old male with type 2 diabetes, retinopathy, renal insufficiency and peripheral arterial disease presented with a surgical wound of 10 days' duration (12.8x5.8cm (74.2cm^2)). Skin changes consistent with chronic venous disease were also present. The patient was being managed with a blood thinner (Warfarin, 10mg) and had fallen, causing a pretibial haematoma which was surgically opened and drained. The UPPER assessment score was 5: unhealthy tissue, pain, poor healing, exudate and smell, and 60% of the ulcer surface was covered by residual haematoma and unhealthy tissue leading to the decision to use WDP to debride. Following debridement once a week with WDP, the ulcer was managed with Betadine gauze dressing (Baxter) primary dressing to reduce bacterial load, and gauze secondary dressing retained with Comprilan compression bandage (BSN Medical), changed once daily. After 18 days the dressing regimen was changed to AMD Foam Dressing with PHMB (Covidien), and Coban2 compression bandage (3M) was used and changed three times per week. Haematoma and unhealthy tissue were formally assessed at week zero and week four. The ulcer surface area covered by haematoma and unhealthy tissue reduced during treatment from 60% to 20%, the ulcer surface area reduced by 81% to 4.0x2.5cm (10.0cm^2), and exudate had reduced at week four. The UPPER score

Fig 5. Patient 3, a 66-year-old male, with a venous leg ulcer (79cm^2) of 23 days' duration on the medial aspect right leg, UPPER score 4. All the wound was covered by slough at week 0 (**a**). The monofilament wound debridement pad removed all slough. Week two (**b**) and week three (**c**) are shown. By week four the ulcer surface area reduced by 98% and the UPPER score reduced to 0 (**d**)

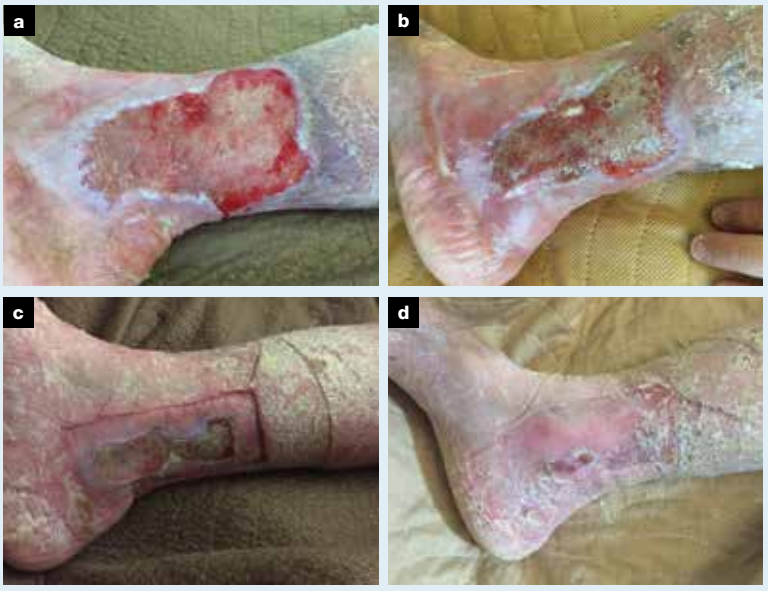
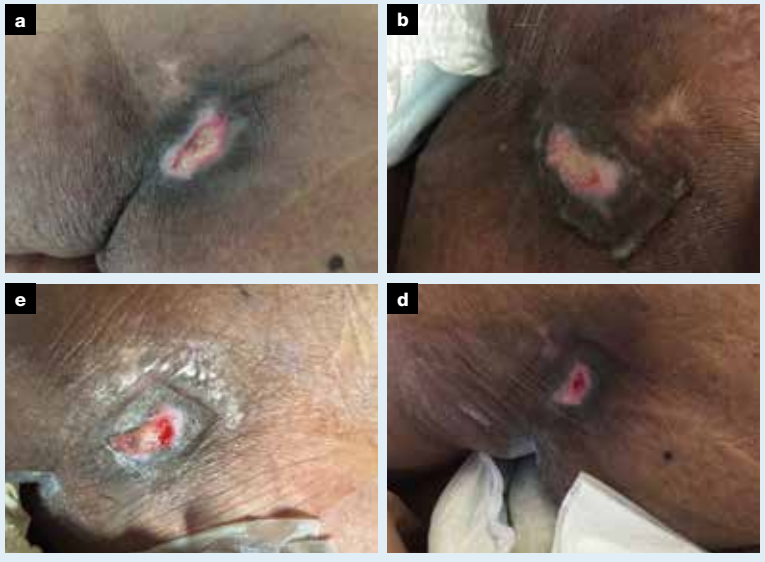


Fig 6. Patient 5, a 78-year-old-female, with a category III pressure ulcer on the sacrum (0.5cm^2), of four weeks' duration; UPPER score 3. Slough covered 60% of the ulcer surface area at week 0 (**a**). The monofilament wound debridement pad effectively removed all slough. Week 1 (**b**) and week 3 (**c**) are shown The ulcer healed completely and the UPPER score was 0 at week 4 (**d**)



reduced to 1 at week four. Fig 8b shows the wound and WDP pad immediately post-debridement and illustrates the effectiveness of WDP. The wound surface is clean with healthy granulation tissue and the haematoma has transferred to the WDP. Procedural pain was assessed by the patient to be at level 0 at both week zero and week four, indicating that the patient sensed no pain during debridement with WDP at the start and end of the treatment.

Discussion

A chronic wound with slough and devitalised tissue is an ideal environment for the proliferation of bacteria that leads to the formation of biofilm.[24] Polymicrobial biofilm is considered to be ubiquitous in chronic wounds,[4,5,25–28] and its removal and management is a critical element of best practice in wound care.[29,30] Once removed, without further management, remaining organisms become active and reform biofilm.[17] The tolerance of biofilm bacteria to antiseptics, antibiotics and the host's defences is reduced by disruption or removal of biofilm, and the reactivation of microbial metabolism in a treatment modality that has been called BBWC, a close analogue of wound bed preparation but with specific focus on biofilm.[17,31] Topical antimicrobial agents applied to these organisms are thereby more able to kill residual organisms and help prevent the reformation of biofilm.[17]

There is a growing body of laboratory and preclinical evidence, and expert opinion supporting the proposition that biofilm delays wound healing, probably by stimulating chronic inflammation that leads to chronically elevated levels of protease and ROS.[32] Chronic wounds such as VLUs, PUs and DFUs exhibit a chronic inflammatory response triggered by the overstimulation of endogenous processes normally controlled in acute wounds.[33–35]

The open wound initially becomes colonised by endogenous and exogenous organisms that quickly establish a biofilm community particularly when the host's immune system may be compromised by the inflammatory nature of the wound.[36,37] The species mix of wound bacteria evolves and fluxes over time becoming more complex.[38,39] The endogenous inflammatory state is then overlaid with additional inflammatory stimulus mediated by biofilm. Evidence consistent with a role for biofilm in stimulating the inflammatory processes of wounds,[4,40,41,42] suggests that this may underpin the chronicity of subclinically infected wounds containing biofilm. Preclinical investigations have shown impaired healing in murine diabetic full-thickness,[9,43,44,45] porcine partial-thickness and full-thickness wounds,[46,47] and equine wounds[48] with biofilm.

The direct effects of biofilm in human clinical wounds remain largely unresolved[49] and based on extrapolated preclinical and laboratory data. Further research is required to resolve this question[37] although surgical wound healing may be delayed by biofilm.[50] The consensus is that biofilm is strongly implicated in the formation and recalcitrance of chronic wounds.

The identification of biofilm in human wounds is challenging and based on clinical observation of secondary effects that may be related to the presence of biofilm.[50] Definitive diagnosis of biofilm in wounds requires a biopsy and light[18] or SEM.[50] Despite this, it is clear that a regimen of wound management that includes debriding slough and biofilm improves wound healing rates,[14,30] a principle that is reflected in wound management guidelines.[29,51–54] Indeed, it has been proposed that clinicians should 'assume all non-healing, chronic wounds that have failed to respond to standard care have biofilm'.[32]

Slough is a substrate upon which biofilm readily forms,[55] particularly in the ideal environment of a chronic wound.[24] In the investigation by James and colleagues into the prevalence of biofilm in wounds,[4] specimens derived from sharp debridement of 50 chronic wounds, including DFUs, PUs and VLUs, were examined. Biofilm was identified in 60% of these wounds. A high proportion of 40 VLUs were shown, by bacterial tag-encoded FLX and Titanium amplicon pyrosequencing and metagenomics, to contain biofilm following removal of surface debris and biopsying the wound bed.[27] In another study,[26] biofilm was identified

Fig 7. Patient 6, an 82-year-old female with a category III pressure ulcer on the sacrum (8.05cm^2) of four weeks' duration; UPPER score 4. Slough covered 70% of the ulcer surface area at week zero (**a**). The monofilament wound debridement pad reduced the ulcer area covered by slough to 30%. The ulcer area reduced to 6cm^2 and the UPPER score reduced to 2 at week four (**b**)

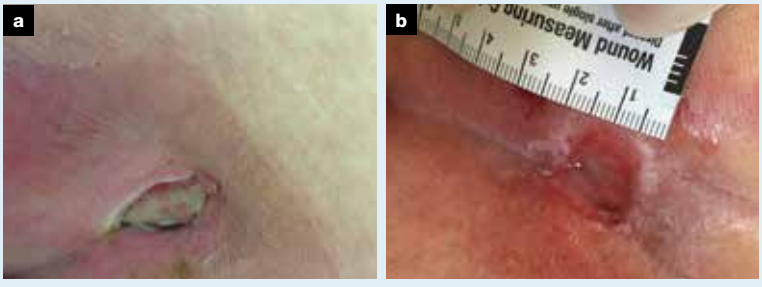
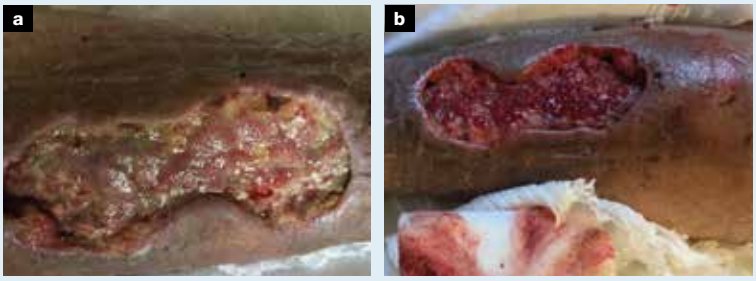


Fig 8. Patient 7, a 55-year-old male with a surgical wound (74.2cm^2) on the pretibial area of four weeks' duration; UPPER score 5. Residual haematoma and unhealthy tissue covered 60% of the wound surface area at week zero (**a**). WDP effectively reduced the wound area covered by haematoma and unhealthy tissue to 20%. The wound area reduced by 81% and the UPPER score reduced to 1 at week four (**b**)



in all biopsies taken from the edge of the ulcer in 65 patients with DFU following cleansing with saline. A recent meta-analysis of nine studies by Malone et al.[5] documented that biofilm was evident in 78% of chronic wounds biopsies. Given the frequency with which slough presents in chronic wounds, it is reasonable to speculate that, in many of the chronic wounds in these studies, biofilm and slough would have coexisted. Wound slough is typically a mixture of plasma proteins, especially fibrin, degraded ECM proteins, especially collagen, planktonic bacteria, and may also include aggregates of biofilm bacteria that may have detached from the wound bed by the autolytic debriding action of proteases.[18,55] Therefore, we speculate that mechanical debridement of slough will remove biofilm associated with slough. Biofilm that forms in a low-shear environment exhibits low tensile strength and breaks easily, whereas biofilm that forms in high-shear environments, such as valves in the cardiovascular system, is notably strong and resistant to mechanical breakage.[56] Hence, wound biofilm forms in a low-shear environment and may be relatively easily removed.

Previously, *in vitro* studies on the antibiofilm function of WDP have demonstrated effective biofilm removal.[57] However, *in vitro* laboratory models of human wounds, particularly chronic wounds, are limited by their inability to recapitulate the complexity of those clinical wounds.[20] Laboratory models may more closely represent the clinical situation when the substrates used in the model are similar to those found in a wound. In this investigation, a validated model that mimics the human clinical wound more closely, by using porcine dermal tissue, was employed.[21] This porcine explant model more closely represents the clinical wound than a model created on laboratory plastic does, and results from it may be extrapolated to the clinical situation with a higher degree of confidence.

Porcine skin is considered a close structural analogue of human skin[20,58,59] and has been used in *in vivo* wound healing studies with a variety of products and agents.[58,60–64] Porcine skin satisfies a number of requirements for a model for studying wound biofilm, including the aforementioned similarity to human skin, control of variables, and its ability to generate reproducible results. Furthermore, a large number of samples can be evaluated at a reasonable cost.[21] The clinical wound was represented by a dermatome 'wound' in the explant exposing dermal tissue, and biofilm was cultured on the exposed dermal surface. This mimics the formation of biofilm on a human wound surface. The model uses antibiotics to kill planktonic organisms while leaving biofilm organisms unaffected, an approach that takes advantage of the property of the EPS that protects biofilm organisms from antibiotics by limiting penetration.[56,65] The model thereby differentiates between planktonic and biofilm organisms.[21] The porcine explanted skin model has been validated and used to evaluate antibiofilm activity for a variety of wound management products[66–68] and for biofilm debridement studies.[69]

In the porcine explanted skin model, we found that WDP removes biofilm and reduces the number of planktonic organisms. This finding is consistent with, and extends, previously-published *in vitro* and *ex vivo* findings for WDP[57] and advances our mechanistic understanding by employing a more clinically relevant substrate for biofilm formation. The outcome was consistent when repeated by the same operator (QY) and when a laboratory technician (QY) and clinical expert (DW) conducted the debridement. In all experiments a similar proportion of biofilm was removed. The outcome was similar when gauze was used, although the application force used were not measured, the investigators noted subjectively that less force was required when using WDP than when using gauze. Reduced force may be important in patient-reported outcome measures (PROMs) such as pain and discomfort. The clinical element of this investigation measured procedural pain during debridement by WDP. Overall, there was little or no change in perceived pain levels at weeks zero and four in the 10 patients evaluated. The clinical cases do not allow direct interpretation of these finding relative to other modes of debridement such as gauze. However, our clinical experience suggests that gauze causes more debridement-related procedural pain than is reported for WDP. *In vitro* studies[57] have further demonstrated a performance advantage for WDP compared with gauze. In these studies the debridement performance of WDP was maintained over 15 repeated debridements using the same pad whereas for gauze, performance declined after only five debridements with the same pad. This suggests that WDP has a higher capacity for debridement than gauze which may also extend to the clinical setting.

All wounds were selected because of a clinically-identified need to debride slough in order to meet the requirements of best practice in wound bed preparation.[51,70] The mean surface area covered by slough in the 10 wounds at week zero was 79%. In every case the surface area of the wound covered by slough at week 0 reduced by week four to a mean of 8%. WDP effectively removed slough and residual haematoma which transferred to the WDP, leaving a clean wound bed. Despite this, slough reformed between treatment episodes and was effectively debrided at each treatment episode. In our experience with these 10 patients, WDP effectively debrided slough and haematoma.

Every wound was judged by the UPPER assessment to have unhealthy tissue, however all improved over four weeks, three achieving complete closure. The amount of exudate reduced in every wound, and UPPER scores fell to 0 in 50%. None of the 10 patients managed in this case series developed infection, and no antibiotics were indicated.

Limitations

The laboratory elements of this study were based on

ex vivo tissue with a monomicrobial biofilm without wound exudate and underlying pathology. The model used is a closer representation of the human clinical wound than a model based on abiotic surfaces is, but direct extrapolation to the clinical wound should be approached with caution. Nevertheless, the controlled conditions allowed comparison with gauze and between debridement effectiveness when two different operators conducted the experiments. The presence of biofilm on WDP was not confirmed but SEM visualisation clearly showed reduced biofilm structures on debrided explants.

The clinical case series in this study was conducted in a limited number of acute and chronic wounds. All observations on the removal of slough, and other endpoints, with the exception of wound surface area, were subjective. The consistency of response to debridement with WDP suggests that the results are likely to be repeated in other wounds although this should be confirmed in further studies. The wounds managed with WDP in the clinic were all affected by loosely-attached sloughy debris. These findings do not therefore apply to wounds affected by more tenacious material or thick eschar tissue requiring debridement.

Biofilm was not specifically evaluated in the clinical wounds but it is reasonable to speculate that biofilm and slough coexist in many wounds where slough is present. Biofilm is a microscopic structure that cannot be visualised, however, biofilm is likely to be present in most chronic wounds based on published findings, even in the absence of slough. These speculations should be confirmed in clinical studies in which biofilm removal is evaluated by quantitative microbiology, using biofilm-specific sample preparation, and light microscopy or SEM visualisation to measure biofilm removal and correlating removal with wound progression. The presence of biofilm on WDP post-debridement and the removal of loosely-attached slough should be demonstrated by microscopic examination of WDP pre- and post-debridement.

Conclusions

Effective debridement requires enough pressure and shear force to remove slough and biofilm. WDP provides an effective solution to the goal of removing loose slough and biofilm, deeply cleaning wounds and removing exudate. Pain and trauma are important in patient QoL. WDP does not induce high levels of procedural pain and any such pain subsided once debridement was complete. These encouraging clinical outcomes provide a mechanistic basis for, and support, the overall effectiveness of a standard of wound care that includes WDP to debride slough from a variety of acute and chronic wounds, and is aligned with the principles of both WBP and BBWC. **JWC**

Reflective questions

- How does biofilm affect wound healing?
- Describe biofilm based wound care?
- What are the advantages and disadvantages of a monofilament debridement pad?

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