Evaluation of the combination of a biofilm-disrupting agent and negative pressure wound therapy: a case series

Objective: Approximately three million people in the US have hard-to-heal pressure ulcers (PUs), including 10% of hospitalised patients. Healing depends on ulcer stage and patient comorbidities. Despite advances in nutrition and wound care, PUs can take months or years to reach complete closure. To date, clinical studies have focused on single modality therapy. However, there is no one therapy that can address all of the deficits in these complex, hard-to-heal wounds. A commonly used treatment for PUs, negative pressure wound therapy (NPWT), has demonstrated improved healing in Stage 3 and 4 PUs. NPWT entails applying suction to a porous sponge fitted into the wound cavity and sealed with an occlusive dressing. Negative pressure facilitates wound healing by removing wound fluid containing harmful proteases, stimulating the formation of granulation tissue and promoting wound contracture. However, it does not affect biofilm formation. We hypothesised that adding an antibiofilm agent might increase the effectiveness of NPWT in recalcitrant PUs.
Method: A prospective case series was conducted in outpatient wound care centres and a skilled nursing facility to examine the combination of a biofilm-disrupting antimicrobial agent (Blast-X, Next Science, US) in combination with NPWT (VAC, 3M, US) in healing and reducing bacterial burden in treatment-resistant pressure ulcers. Patients consented to application of the antibiofilm agent and NPWT three times per week for four weeks. The wounds were measured, imaged for bacteria and tested for host and bacterial protease activity weekly.
Results: Of the 10 patients, four dropped out of the study before the end of the four weeks. Of the remaining six, four patients experienced a reduction in wound surface area and volume, reduced protease activity and lower bacterial levels.
Conclusion: The results of this study showed that multimodal therapy, including NPWT and biofilm disruption, may restart the healing of stagnant treatment-resistant PUs.
Declaration of interest: This research was sponsored by a grant for Next Science Inc. Matthew Mynti is the Chief Technology Officer for Next Science. The authors have no other conflicts of interest.

biofilm • dressing • negative pressure wound therapy • pressure injuries • pressure ulcers • wound • wound care • wound healing • wounds

Approximately three million people in the US have pressure ulcers (PUs) at an annual cost of approximately $10 billion USD. The Agency for Healthcare Research and Quality (AHRQ) estimated that the cost of treating a PU in 2014 ranged from $20,900–151,700 USD per ulcer.[1]

Once ulcers develop, they heal slowly. A recent article evaluating the medical records of over 100,000 patients reported that only 14.9% of pressure ulcers healed by 12 weeks.[2] A variety of local and systemic factors contribute to delayed wound healing in PUs, including age, wound size and depth, bacterial burden, elevated matrix metalloproteases, unrelieved pressure, and comorbidities such as diabetes, renal disease, malnutrition and obesity.[2–5]

The most commonly used staging system for PUs (also known as pressure injuries), published by the National Pressure Injury Advisory Panel (NPIAP), designates full-thickness ulcers as Stage 3 or 4.[6] However, in the absence of underlying osteomyelitis, the treatment for Stages 3 and 4 is similar. To date, treatment strategies have focused on single therapeutic interventions. A clinical practice guide published by the American College of Physicians recommends three modalities: protein supplementation, foams or hydrocolloids and electrical stimulation.[7]

In addition, several studies suggest negative pressure wound therapy (NPWT) promotes granulation tissue formation in Stage 3 and 4 PUs.[8] NPWT involves the application of suction to a porous sponge fitted into the wound cavity and sealed with an occlusive dressing. Negative pressure facilitates wound healing by removing wound fluid containing harmful proteases, stimulating the formation of granulation tissue and promoting wound contracture.[9] However, it does not affect biofilm formation. Bacteria are known to impede wound healing. A prospective study using fluorescence bacterial imaging demonstrated that large amounts of bacteria accumulate under the dressing.[10] We hypothesised that adding an antibiofilm agent may increase the effectiveness of NPWT in recalcitrant PUs.

Thomas E Serena[1], MD, FACS, FACHM, MAPWCA, Founder and Medical Director*; **Omar Jalodi**[1], MD, Natrox Wound Care Fellow; **Laura Serena**[1], LPN, Vice President Research Operations; **Keyur Patel**[2], DO, Partner D&P; **Matthew Mynti**[3], PhD, Chief Technology Officer for Next Science
Corresponding author email: serena@serenagroups.com
1 SerenaGroup Research Foundation, Cambridge, MA US. **2** D&P Medical Group, Pittsburgh PA. **3** Next Science Inc.

BlastX (Next Science, US), the antimicrobial gel chosen for this prospective case series, contains a broad-spectrum antimicrobial agent, benzalkonium chloride, that kills Gram-positive and Gram-negative bacteria and fungi. An antimicrobial agent alone will kill planktonic bacteria but will have little effect on bacteria protected in the extracellular polymeric substance (EPS) of a biofilm. In order to solve this problem, two of the ingredients in the antimicrobial gel, citric acid and sodium citrate, attach to the metallic bonds that make the EPS insoluble and bind the metallic links holding the polymers together, weakening the EPS structure and allowing it to go into solution. Removing the EPS then exposes the bacteria to bacterial wall lysis by the benzalkonium chloride, destroying the bacteria in the biofilm. In clinical trials, this biofilm-disrupting agent (BDA) promoted healing in hard-to-heal wounds.[11,12]

In the lead author's experience, the effective treatment of PUs requires multimodal therapy; however, no studies to date support using more than one product at a time. This prospective trial sought to evaluate the combination of two commonly prescribed treatments for hard-to-heal wounds.

Method

This case series is taken from a prospective, open-label, multicentre study (clinicaltrials.gov #NCT04265170) which assessed adult (>18 years of age) patients with longstanding (>3 months) Stage 3 or 4 PUs without osteomyelitis. A total of three sites participated in this study: a free-standing research site, a hospital outpatient clinic and a skilled nursing facility. All the patients signed an Institutional Review Board (IRB)-approved informed consent before any study-related procedures.

Inclusion criteria

- Adult patients with a full-thickness PU, Stage 3 or 4, without exposed bone, of one month or more in duration and located on the trunk (sacral, trochanteric, ischial or posterior heel)
- A signed and dated informed consent form
- Patient is able to comply with instructions and scheduled visits
- PU surface area >2cm^2 and <100cm^2
- The patient is a candidate for NPWT

Exclusion criteria

- Patient or caregiver is unable to manage VAC device OR the patient cannot return for VAC dressing changes OR the patient does not qualify for home health visits
- Patient has major uncontrolled medical disorders such as serious cardiovascular, renal, liver or pulmonary disease, lupus, palliative care or sickle cell anaemia
- Patient currently being treated for an active malignant disease or subject with history of malignancy within the wound
- Patient has other concurrent conditions that in the

Table 1. Patient demographics

Patient	Age (years)	Gender	Ulcer duration (weeks)	Mini nutritional assessment score	Comorbidities (major conditions listed)
1	67	Female	48	7	Type 2 diabetes, multiple trauma following a motor vehicle accident with paraplegia, permanent colostomy, hypothyroidism, anaemia of chronic disease
2	70	Female	156	7	Multiple sclerosis with generalised weakness, recent bilateral hip fractures, overactive bladder
3	70	Male	68	8	Multiple sclerosis with generalised weakness, wheelchair-bound, obesity
4	46	–	58	9	–
5	59	Female	20	11	Right above-knee amputation, chronic general body pain
6	22	Male	60	12	Paraplegia, muscle spasticity, anaemia of chronic disease, neuropathy
7	67	Female	52	13	Diabetes, multiple trauma following motor vehicle accident, paraplegia, history of perforated bowel with ileostomy, history of deep venous thrombosis with caval filter hypothyroidism, anaemia of chronic disease
8	67	Male	8	10	Paraplegia following motor vehicle accident
9	78	Female	104		Diabetes, peripheral arterial disease, chronic obstructive pulmonary disease, dysphagia, encephalopathy
10	66	Female	36	8	Generalised weakness, dysphagia, coronary artery disease, hypertension, incontinence, schizoaffective disorder, lower extremity contractures, seizure disorder

opinion of the investigator may compromise patient safety
- Known contraindications to VAC or BlastX
- Known allergies to any of the BlastX components
- Concurrent participation in another clinical trial that involves an investigational drug or device that would interfere with this study
- Patient is pregnant or breastfeeding
- Patient with a history of >2 weeks' treatment with immunosuppressants (including systemic corticosteroids >10mg daily dose), cytotoxic chemotherapy, or application of topical steroids to the ulcer surface within one month before first screening visit, or who receive such medications during the screening period, or who are anticipated to require such medications during the course of the study
- Index ulcer has been previously treated with tissue engineered materials (e.g., Apligraf or Dermagraft) or other scaffold materials (e.g., Oasis, Matristem) within the last 30 days preceding the first treatment visit
- MNA malnutrition indication score <17
- Patient does not have adequate four-week historical data on comparison in change of wound measurements, photographs, costs and supplies used.

Procedure

A history and physical examination were performed, including an in-depth ulcer history. The ulcer was then staged, using the NPIAP staging system,[13] and the wound surface area, depth and volume were measured, using digital photographic planimetry (ARANZ, New Zealand). Offloading techniques were reviewed with patients and a mini nutritional assessment (MNA)[14] performed. Wound care was standardised between all three sites. The ulcers were debrided at the bedside. Bacterial load was assessed with fluorescence imaging, using the MolecuLight Procedure (MLiX) (MolecuLight Inc., Canada), a validated procedure that detects bacterial load greater than or equal to 1×10^4 colony forming units (cfu)/g.[15] In addition, wound swabs were tested for bacterial protease activity (BPA) at the bedside, using the WoundChek Bacterial Status kit (WoundChek Labs, UK).[16] A wound swab was also taken to analyse host proteases using the WoundChek Protease Status kit (WoundChek Labs, UK).[17]

The reticulated open cell foam dressing for NPWT (VAC, 3M, US) was cut to fit the wound cavity. Before placing it into the ulcer, the BDA was applied directly onto the foam. The foam with BDA was left in the wound for five minutes. (The remainder of the NPWT dressing was placed during the dwell time.) The sponge was then covered with an occlusive dressing and the suction apparatus attached. The pump was set at –125mmHg of negative pressure. The combination dressings were changed three times per week. The patients returned to the clinic weekly for four weeks for examination, photography, wound measurement, MLiX and protease testing.

Fig 1. Wound surface area reduction

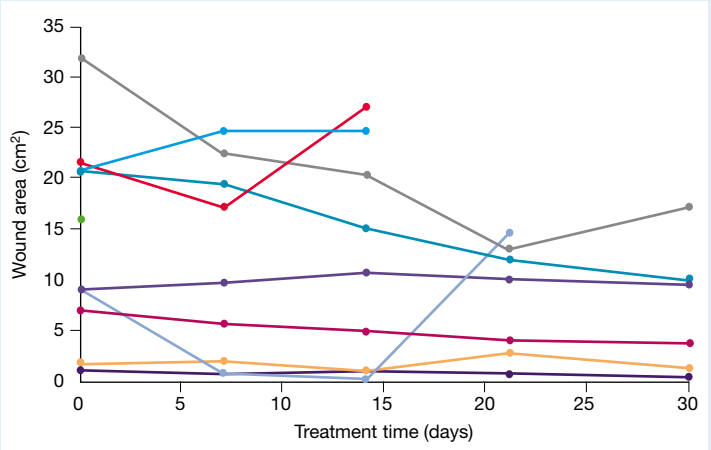


Fig 2. Improvement in wound surface area for the enrolled population and for patients completing four weeks of treatment (responders)

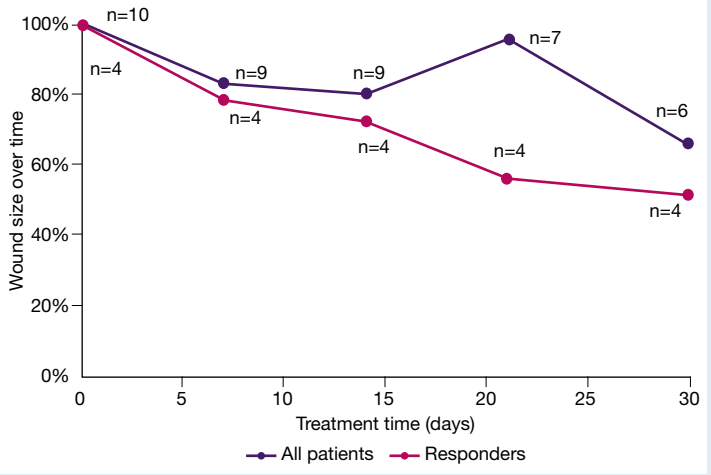


Fig 3. Host matrix metalloprotease (MMP) activity for the enrolled population and for patients completing four weeks of treatment (responders)

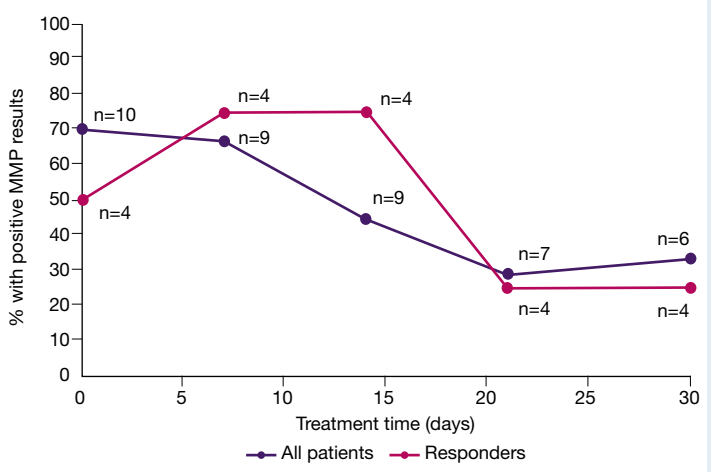


Fig 4. Bacterial protease activity (BPA) for the enrolled population and for patients completing four weeks of treatment (responders)

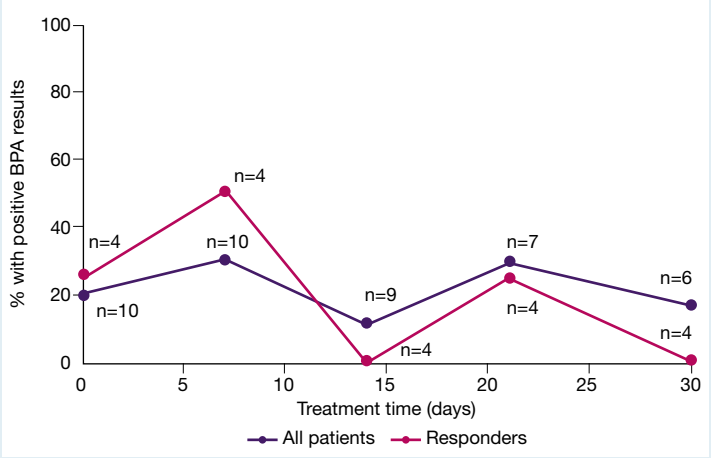
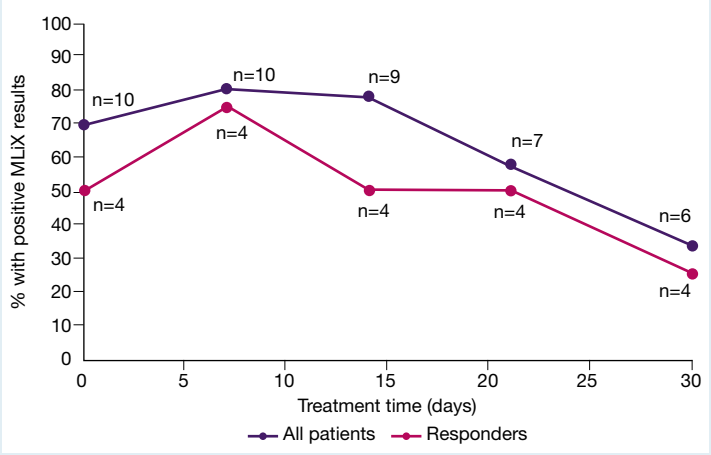


Fig 5. Bacterial burden changes over time for the enrolled population and for patients completing four weeks of treatment (responders)



Results

A total of 10 patients were enrolled prospectively between November 2019 and January 2020. Patient demographics are shown in Table 1. All had failed at least four weeks of NPWT before enrolment, although this was not part of the inclusion criteria. Of these patients, four dropped out over the course of the study, of which one patient dropped out before day seven due to a poor seal with the NPWT. A further two patients dropped out before day 21—one could not tolerate NPWT and a second required hospital admission. The final patient dropped out after worsening of the wound, necessitating operative debridement.

Primary endpoint (wound size)

The primary endpoint, reduction of wound surface area over four weeks, is an established surrogate endpoint.[18] For PUs, a positive healing trajectory is a wound surface area reduction of 20% or greater.[19] Fig 1 shows the wound areas for all patients over time.

Of the ten enrolled patients, four (40%) had positive results (closing by >20% in four weeks). Of the patients who completed the entire course of study, four of the six (67%) had a positive result. The average wound surface area reduction was 49% (Fig 2).

Secondary endpoints

Change in host proteases during therapy was a secondary endpoint for the trial. The WoundChek Protease Status (WCPS) (WoundChek Inc., UK) point-of-care test detects levels of matrix metalloproteases (MMPs)[2,8,9] and human neutrophil elastase. Fig 3 graphically represents the reduction in protease activity during the four-week treatment period. The sample size is too small for statistical analysis.

Bacterial protease activity during the trial did not demonstrate any trends in this small sample (Fig 4). The WoundChek Bacterial Protease Status (WCBS) (WoundChek Inc., UK) point-of-care test detects bacterial proteases for five common pathogens in hard-to-heal wounds.

The MLiX procedure results demonstrated a trend toward a reduction in bacterial burden with complete elimination of bacterial fluorescence in 30% of the patients. The graph in Fig 5 represents the change in bacterial fluorescence over four weeks.

Discussion

NPWT is a mainstay in the treatment of Stage 3 and 4 PUs, with a body of evidence demonstrating increased granulation tissue formation.[8] However, NPWT, like all other therapies used to treat PUs, is not always effective. No one treatment can address all the deficits in a hard-to-heal wound. It has been shown that NPWT has little or no effect on the bacterial burden in the wound bed.[20] Its effect on biofilm has not been studied. The addition of an antimicrobial to NPWT is not a new concept—instillation therapy (NPWTi) decreases bacterial load.[21] The NPWT device is modified to allow a liquid, such as normal saline, to flow into the wound cavity. After a dwell time of several minutes, the fluid is then removed and negative pressure applied. The process is repeated three or four times per day. At present, NPWTi use is restricted to the inpatient setting.

It follows that adding an antimicrobial with biofilm-destroying capabilities to NPWT in the outpatient setting may improve healing outcomes. The biofilm disrupting agent BlastX contains ingredients that disrupt the EPS holding the biofilm together and protecting the resident bacteria. Once the EPS is deconstructed, the antimicrobials, in combination with the osmotic pressure, kill the bacteria and reverse the biofilm-induced metabolic wound changes. In this way BDAs promote healing.[22–24]

The results of this prospective study suggest that the combination of a BDA with NPWT may restart the healing process in treatment-resistant Stage 3 and 4 PUs. In patients who completed the study, four of the

six responded to the combination therapy after previously failing NPWT alone. The reduction in wound area in the responders correlated with a reduction in bacterial burden as evidenced by fluorescence imaging (Fig 6). The small study was not powered to show statistical significance. In addition, previous research using the MLiX procedure revealed the build-up of bacteria in the NPWT sponges.[10] We observed that in most cases the fluorescence disappeared from the sponge after two weeks of BDA–NPWT (Fig 7). The significance of this finding is unclear and requires further study.

The data also demonstrated a 50% reduction in host proteases. Previous research has reported an elevation in MMPs associated with NPWT.[3] The fall in MMPs seen here is not statistically significant but the trend toward reduced inflammation is clear. The decreased MMPs may reflect the decrease in bacterial burden, although further investigation in a larger group is needed. The lack of change in bacterial protease activity over time is difficult to explain. The study coordinators suggested that obtaining the swabs after debridement early in the study may have skewed the results.

Limitations

The small number of patients enrolled is a major limitation of this study. The sample size prohibited statistical analysis of the healing, bacterial burden and protease data. The high drop-out rate, although not unexpected in this population, compounded the limitations related to study size. However, the trends seen in this investigation prompted the investigators to expand enrolment. Once the COVID-19 crisis clears, enrolment will resume.

Conclusion

The results from this small prospective trial suggest that the combination of a BDA and NPWT may jump start healing in treatment-resistant PUs. Further research is warranted. JWC

Acknowledgments: The authors would like to thank 3M and Next Science for providing NPWT devices and BlastX.

Fig 6. Standard photograph at day zero (**a**). MLiX image at day zero—the red indicates the presence of bacteria in the wound and on the periwound skin (**b**). MLIX image day 30—fluorescence has disappeared (**c**). Standard photograph day 30—reduction in wound surface area of 50% (**d**)

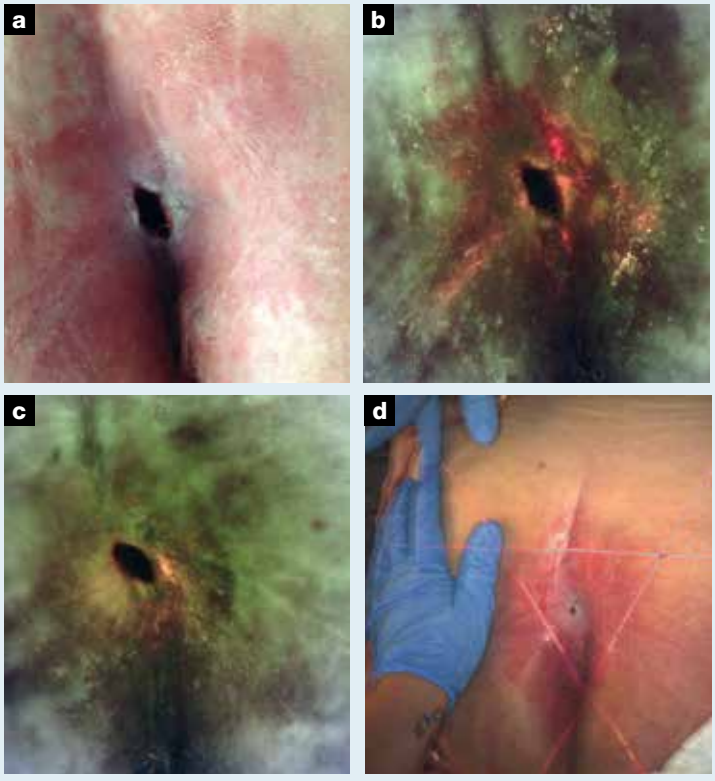
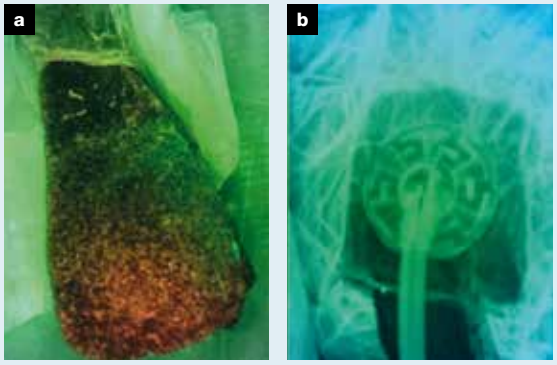


Fig 7. Disappearance of fluorescence in the sponge after treatment: on the left red fluorescence indicating bacteria in the sponge (**a**). No fluorescence at end of treatment (**b**)



References

1 Agency for Healthcare Research and Quality. Preventing pressure ulcers in hospitals. 2014. https://www.ahrq.gov/patient-safety/settings/hospital/resource/pressureulcer/tool/pu1.html (accessed 1June 2020)
2 Kyu Cho S, Mattke S, Gordon H et al. Development of a model to predict healing of chronic wounds within 12 Weeks. Adv Wound Care 2020; 9(9):516–524. https://doi.org/10.1089/wound.2019.1091
3 Nwomeh BC, Yager DR, Cohen IK. Physiology of the chronic wound. Clin Plast Surg 1998; 25(3):341–356
4 Brem H, Tomic-Canic M, Tarnovskaya A et al. Healing of elderly patients with diabetic foot ulcers, venous stasis ulcers, and pressure ulcers. Surg Technol Int 2003; 11:161–167
5 European Pressure Ulcer Advisory Panel, National Pressure Ulcer Advisory Panel. Treatment of pressure ulcers: quick reference guide. 2009. https://gneaupp.info/wp-content/uploads/2014/12/pressure-ulcer-treatment.pdf (accessed 8 December 2020)
6 National Pressure Injury Advisory Panel. Pressure injury stages. 2016. https://npiap.com/page/PressureInjuryStages (accessed 1 June 2020)
7 Qaseem A, Humphrey LL, Forciea MA et al.; for the Clinical Guidelines Committee of the American College of Physicians. Treatment of pressure ulcers: a clinical practice guideline from the American College of Physicians. Ann Intern Med 2015; 162(5):370–379. https://doi.org/10.7326/M14-1568
8 Gupta S, Ichioka S. Optimal use of negative pressure wound therapy in treating pressure ulcers. Int Wound J 2012; 9(1):8–16. https://doi.org/10.1111/j.1742-481X.2012.01012.x
9 Orgill DP, Manders EK, Sumpio BE et al. The mechanisms of action of vacuum assisted closure: more to learn. Surgery 2009; 146(1):40–51. https://doi.org/10.1016/j.surg.2009.02.002
10 Raisman, R. Fluorescence imaging guided dressing change frequency during negative pressure wound therapy: a case series. J Wound Care

Reflective questions

- Are some wounds recalcitrant to negative pressure wound therapy (NPWT) due to the presence of biofilm? If so, what is the mechanism behind this?
- What is the significance of bacterial fluorescence in the NPWT dressing?
- How can antibiofilm agents also reduce host protease levels?
- How can the effectiveness of NPWT be improved by combining it with antimicrobial agents?

2019; 28(Sup9):S28–S37 https://doi.org/10.12968/jowc.2019.28.Sup9.S28
11 Wolcott R. Disrupting the biofilm matrix improves wound healing outcomes. J Wound Care 2015; 24(8):366–371. https://doi.org/10.12968/jowc.2015.24.8.366
12 Kim D, Namen IW, Moore J et al. Clinical assessment of a new biofilm disrupting agent for the management of chronic wounds compared with standard of care: a therapeutic approach. Wounds 2018; 30(5):120–130
13 Edsberg LE, Black JM, Goldberg M et al. Revised National Pressure Ulcer Advisory Panel pressure injury staging system. J Wound Ostomy Continence Nurs 2016; 43(6):585–597. https://doi.org/10.1097/WON.0000000000000281
14 Huhmann MB, Perez V, Alexander DD,Thomas DR. A self-completed nutritional screening tool for community dwelling older adults with high reliability: a comparison study. J Nutr Health Aging 2013; 17(4):339–344. https://doi.org/10.1007/s12603-013-0015-x
15 Serena TE, Harrell K, Serena L, Yaakov RA. Real-time bacterial fluorescence imaging accurately identifies wounds with moderate-to-heavy bacterial burden. J Wound Care 2019; 28(6):346–357. https://doi.org/10.12968/jowc.2019.28.6.346
16 Serena T, Bayliff S, Brosnan P. Bacterial proteases: a marker for a 'state of pathogenesis' in chronic wounds. Poster presented at: Symposium of Advanced Wound Care (SAWC), Las Vegas, NV, US, 24–25 September, 2015
17 Serena TE, Cullen BM, Bayliff SW et al. Defining a new diagnostic assessment parameter for wound care: elevated protease activity, an indicator of nonhealing, for targeted protease-modulating treatment. Wound Repair Regen 2016; 24(3):589–595. https://doi.org/10.1111/wrr.12431
18 Gould LJ, Liu J, Wan R et al. Evidence supporting wound care end points relevant to clinical practice and patients' lives. Part 3: The patient survey. Wound Repair Regen 2020. https://doi.org/10.1111/wrr.12872
19 Atkin L, Bućko Z, Montero EC et al. Implementing TIMERS: the race against hard-to-heal wounds. J Wound Care 2019; 28(Sup3): S1–S49
20 Patmo AS, Krijnen P, Tuinebreijer WE, Breederveld RS. The effect of vacuum-assisted closure on the bacterial load and type of bacteria: a systematic review. Adv Wound Care 2014; 3(5):383–389. https://doi.org/10.1089/wound.2013.0510
21 Goss SG, Schwartz JA, Facchin F et al. Negative pressure wound therapy with instillation (NPWTi) better reduces post-debridement bioburden in chronically infected lower extremity wounds than NPWT alone. J Am Coll Clin Wound Spec 2014, 4:74–80. https://doi.org/10.1016/j.jccw.2014.02.001
22 Attinger C, Wolcott R. Clinically addressing biofilm in chronic wounds. Adv Wound Care 2012; 1(3):127–132. https://doi.org/10.1089/wound.2011.0333
23 Johani K, Malone M, Jensen S et al. Microscopy visualisation confirms multi-species biofilms are ubiquitous in diabetic foot ulcers. Int Wound J 2017; 14(6):1160–1169. https://doi.org/10.1111/iwj.12777
24 Snyder RJ, Bohn G, Hanft J et al. Wound biofilm: current perspectives and strategies on biofilm disruption and treatments. Wounds 2017; 29(6): S1–S17