WORLD UNION OF WOUND HEALING SOCIETIES
CLINICAL REPORT

Innovations in hard-to-heal wounds

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A well-established practical and predictive measure of complete wound healing over the longer term (24 weeks) is per cent change in wound area over the first 4 weeks[1]. However, certain conditions have the potential to delay healing and signs may indicate stalled healing: infection, ischaemia, or abnormal inflammation, with impaired inflammatory response often being self-perpetuating[2]. Non-healing wounds contain microbial, biochemical or cellular abnormalities that delay healing progression[3], with biofilm presence often implicated[4].

THE IMPACT OF HARD-TO-HEAL WOUNDS

With the prevalence and incidence of wounds increasing due to aspects such as an ageing population and comorbidities including diabetes[5], a high economic and humanistic burden is incurred (Table 1). In hard-to-heal wounds, this burden is compounded: complications occur, patients become more dependent and costs increase, typically driven by a need for increased healthcare professional time[6].

Table 1 | Cost of wounds to the healthcare system, society and patient

Category	Examples
Economic[5,6]	
Hospital and other facility costs	Inpatient hospitalisation and readmissions, outpatient clinic visits
Specialist care or treatments	e.g. surgical procedures such as amputation
Healthcare professional time	e.g. for dressing changes, community care visits, travel
Materials, interventions, specialist equipment	Dressings, devices, medicines (e.g. antibiotics), other disposables, orthotics
Assessment tools	Diagnostic equipment, laboratory testing
Patient out-of-pocket payments	e.g. travel costs
Lost productivity	Patient or carer lost work time
Health-related quality of life[7]	
Physical wellbeing	Pain, impaired mobility and functioning, poor nutrition or sleep
Mental wellbeing	Depression, anxiety
Psychosocial wellbeing	Social isolation, difficulty with social interactions
Spiritual/cultural wellbeing	Difficulty connecting with one's self and others, impact of cultural nuances and personal values on physical, mental and psychosocial wellbeing

WOUND HEALING IS COMPLEX AND MULTI-FACETED

Numerous factors may impact the complex and multifaceted process of wound healing[6], including issues associated with the patient (i.e. comorbidities and medication), their wound (e.g. size, duration, location), clinical service delivery (i.e. competency of the healthcare professional) or various biophysiological factors (Figure 1)[2].

Recently, certain factors have gained recognition due to their considerable influence on outcomes. Biofilm is present in the majority of chronic wounds (at least 60%)[9], they are often a precursor to overt infection with increasing tolerance to antimicrobial agents, including antibiotics, and tend to form where exudate is not under control[10–12].

Biofilm can impair healing by stimulating an inflammatory response that leads to abundant neutrophils and macrophages (in an attempt to remove the biofilm),

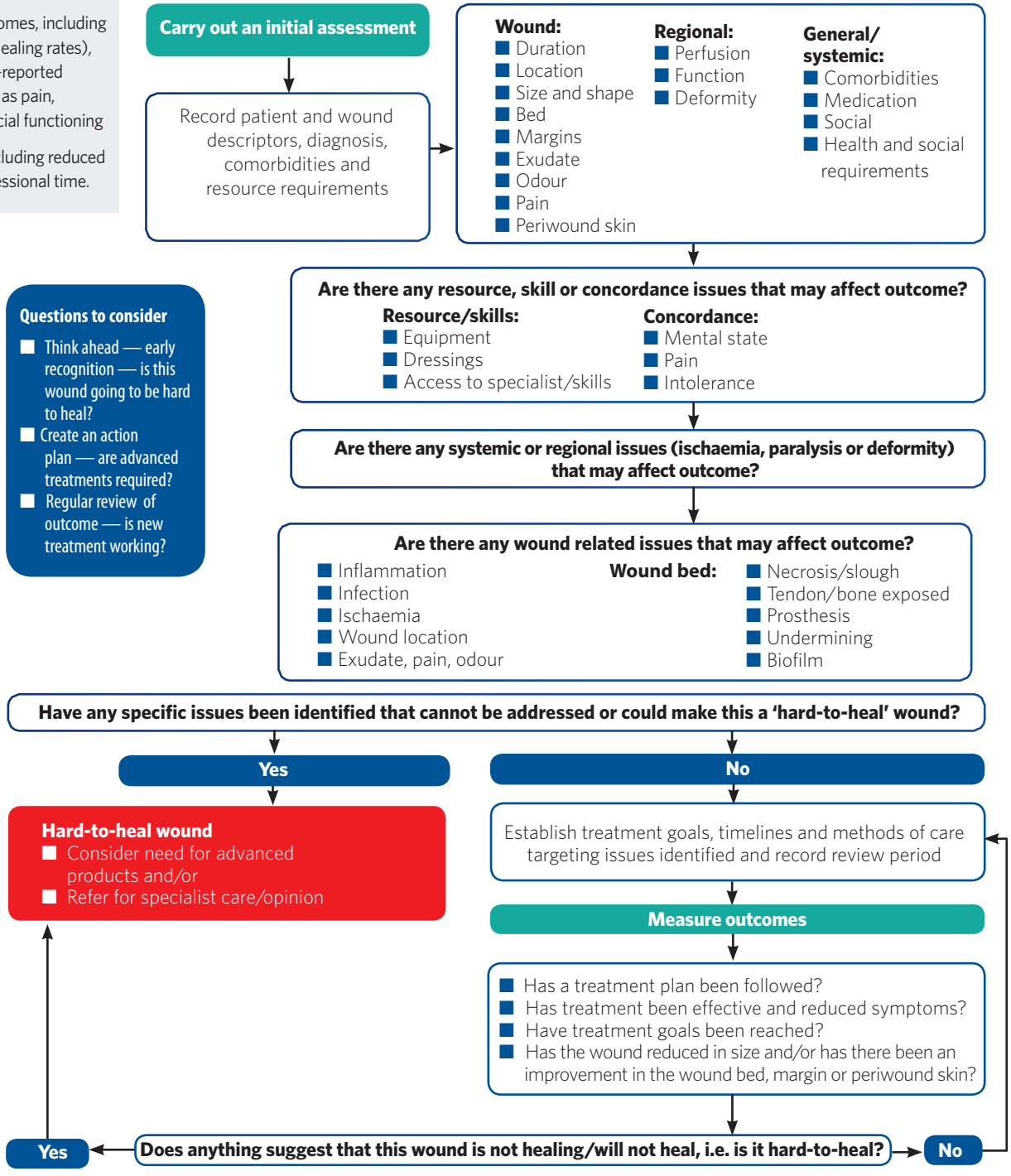
Box 1: Advances in care

Changes to diagnosis and treatment of hard-to-heal wounds, using advanced technologies[8], could lead to improvements in:

- Patient quality of life
- Symptom control, where they are present
- Long-term outcomes, including traditional (i.e. healing rates), but also patient-reported outcomes, such as pain, malodour, or social functioning
- Cost of care, including reduced healthcare professional time.

which secrete high levels of reactive oxygen species and proteases (i.e. matrix metalloproteinases [MMPs])[4]. Numerous studies have shown the presence of biofilm promotes a sustained inflammatory state and delays wound healing[13], and elements of this response may actually facilitate their development[14]. Biofilm provides protection to the contained microorganisms and increases exudate production[15], supporting the inhibition of tissue granulation and epithelialisation.

Figure 1 | Carrying out an initial assessment for recognising hard-to-heal wounds. Adapted from Vowden, 2011[2]



Box 2: How does biofilm protect microorganisms?

Biofilm enhances the tolerance of microbes to factors that would easily kill the same microbes when growing in an unprotected state, including the immune system, antimicrobials and environmental stressers[4].

The biofilm matrix (or the extracellular polymeric substance) forms a physical barrier preventing removal of waste products from around the microbial cells[38] creating regions of metabolic waste and low oxygen tension, and blocking large molecules such as antibodies and inflammatory cells from penetrating deep into the biofilm matrix[4].

These anoxic cores influence surrounding microbial cells, providing unique cooperative and protective effects (such as secretion of protective enzymes that protect neighbouring non-antibiotic-resistant microorganisms), and making them dormant (metabolically quiescent) and so more tolerant to antibiotics and biocides[39].

Infection and biofilm

Microorganisms are commonly divided into two distinct phenotypes: single cells (i.e. planktonic) or sessile aggregates (i.e. the biofilm mode of growth). Research into bacterial pathogenesis has previously focused primarily on acute — or planktonic — infections, which result from invasion by free-floating, solitary microorganisms, as has the development of prevention and treatment control measures. However, a new category of chronic infection caused by microorganisms growing as biofilm has become an increasingly important focus in wound care[16].

Hard-to-heal wounds are often chronically infected, producing a distinct pattern of growth associated with biofilm[17], which can be 500 to 5,000 times more tolerant to antimicrobials[18]. Chronic biofilm-based infections:

- Have a slower progression than acute infections
- Are characterised by an adaptive inflammatory response
- Are typically extremely resistant to antibiotics and many other conventional antimicrobial strategies
- Have an innate ability to evade the host's defences[16].

Regardless of phenotype, microbial cells have multiple mechanisms to attach to specific host epitopes[19–21]. Within minutes, over 800 biofilm genes may be expressed[20], providing genetic capability for microbial cells to communicate and co-operate (quorum sensing)[22–24], develop protection (self-secreted matrix polymers)[25,26], and secrete molecules preventing host immunity counter measures[27–29] (Figure 2).

Figure 2 | Molecular, biochemical and cellular components of biofilm[17, 30–37]

At a molecular level biofilms require:

- Attachment
- Rapid development of a microcolony
- Secretions of molecules to produce host cells senescence (loss of cells' power to divide and grow)
- Hyper-inflammation to produce plasma exudate; achieved via release of outer membrane vesicles, release of planktonic cells, and subversion of host immunity

At a biochemical and cellular level, biofilms produce:

- Excessive neutrophils (i.e. lysozyme, myeloperoxidase, Cathepsin G, etc.)
- Elevated pro-inflammatory cytokines (IL-1, IL-8, gamma interferon, TNF-α)
- Elevated MMPs (MMP-2, MMP-8, MMP-9, elastase)

Uncontrolled exudate

Poorly managed wound exudate can harm the wound healing trajectory, as it can slow down or prevent cell proliferation, interfere with growth factor availability, or contain high levels of proteases and pro-inflammatory cytokines that degrade the host extracellular matrix[40].

Chronic wound fluid also challenges skin integrity around the wound — intact periwound skin has been shown to have a five-fold decrease in barrier function simply by virtue of the underlying tissue inflammation[41]. Moreover, prolonged moisture exposure leads to maceration[42], which increases likelihood of friction and shear. In combination with the decrease in periwound barrier function, maceration increases the risk of chemical irritation from inflammatory exudate and bacterial invasion.

INNOVATIVE APPROACHES TO TREAT HARD-TO-HEAL WOUNDS

Innovation in assessment, diagnostics and treatment

The key to effective diagnostics is how efficiently they are used in practice. Although point-of-care technologies, such as a test allowing practitioners to measure elevated protease activity[43], may offer the best opportunities for real-time decision-making, these have yet to be implemented within daily care.

Box 3: The role of microbial wound mapping

Handheld microbial auto-fluorescence technology that 'maps' microbial distribution[45]. Microbial wound mapping enabling targeted debridement to reduce microbial load, measurement of periwound temperature, and assessment of pain patterns allow for reliable stratification of patients to receive appropriate antimicrobial treatment[45,46]

- Forward-looking infra-red thermography for detecting heat due to inflammation[47]
- Colorimetric detection of host inflammatory markers of infection[48]
- Wound dressings that respond to bacterial infection mediators[49] or wound parameters such as pH[50]

The TIME framework (Tissue, Infection/Inflammation, Moisture, Edge of wound) is a well-established assessment and management method, and remains the typical wound bed preparation paradigm in practice[44]. Since its original presentation, substantial developments in our understanding of wound care have occurred; in particular, regarding the bacterial continuum through contamination, colonisation and infection, as well as the presence of biofilm. TIME remains relevant, but there is a need to ensure these developments are incorporated into assessments[15].

Other diagnostics are in development with the potential to address gaps, providing further objective means to improve the healing trajectory.

An innovative, advanced strategy that targets local barriers to healing

Management of microbial load is vital in the prevention of infection. Moreover, although moist wound healing strategies are no more likely to promote infection than earlier dry wound healing strategies[51], the combination of pooled exudate associated with fully saturated dressings[9] and the corrosive nature of chronic wound exudate may be linked to biofilm development and resulting infection.

Prior to the discovery that keeping wounds moist would improve healing[52], the traditional approach was to soak up fluid and leave the wound to dry. As understanding increased regarding the optimum wound healing environment, the first film dressings with polyurethane technology were developed[53], followed by alternatives such as alginates and hydrocolloids[54], and later, Hydrofiber™ Technology.

As shown in Figure 3, since the first Hydrofiber™ Technology was developed 20 years ago, various products have been developed based on its unique physicochemical properties.

Box 4: What is Hydrofiber™ Technology and how does it work?

- **What is it?**
  - Hydrofiber™ Technology is a soft, conformable material composed of sodium carboxymethycellulose, which can absorb a large amount of wound fluid that is transformed into gel to create a moist environment. While Hydrofiber™ Technology is neither hydrocolloids nor alginates, it incorporates benefits from both while addressing their weaknesses, including cohesive gelling and aggressive adhesion (as demonstrated *in vitro*)[55]
- **Mode of action**
  - Hydrofiber™ Technology allows rapid permeation of fluid and full expansion of fibres, creating a gel that resists wicking within fibres and prevents wicking between fibres, by way of gel blocking (as demonstrated *in vitro*)[56]. This gel provides intimate contact with the wound bed, filling 'dead space' where microbes could grow. Excess fluid is retained, locking in harmful components such as endogenous proteinases and exogenous microorganisms found in wound exudate and reducing transmission to the surrounding skin[57].

Figure 3 |The evolution of Hydrofiber™ Technology in AQUACEL™ dressings

Hydrofiber Technology	AQUACEL
Plus ionic silver for antimicrobial action	AQUACEL Ag
With strengthening fibre	AQUACEL WSF (Ribbon)
Combined with hydrocolloid technology	AQUACEL Surgical
Combined with hydrocolloid technology and ionic silver for antimicrobial action in surgical care	AQUACEL Ag Surgical
With increased strength, absorbency, and wear time	AQUACEL Extra
A comfortable and simple foam dressing	AQUACEL Foam
With increased strength, absorbency, and wear time, plus ionic silver	AQUACEL Ag Extra
A comfortable and simple foam dressing with ionic silver	AQUACEL Ag Foam
Plus ionic silver for antimicrobial action in burn care	AQUACEL Ag Burn
With Ag+technology and synergistic anti-biofilm components — ethylenediaminetetraacetate and benzethonium chloride — to target biofilm, manage exudate and reduce risk of infection	AQUACEL Ag+ Extra

Table 2 provides an overview of in vitro, in vivo *and real-life, clinical evidence for the recent addition of Ag+ Technology to Hydrofiber™ Technology.*

Table 2 | Available evidence for the combination of Hydrofiber™ and Ag+ Technology

Year	
2014[58–60]	***In vitro* data showed:** ■ Biofilm eradication with a single dressing application — mature *Pseudomonas aeruginosa* (4 days) and community-associated methicillin-resistant *Staphyloccocus aureus* (CA-MRSA) biofilm (5 days) ■ Bacterial counts significantly reduced ($p < 0.05$), and improvements in bacterial burden/healing in polybacterial wounds ($p < 0.05$) ■ Ability to prevent reformation (bioburden control after a simulated contamination event) ■ Hydrofiber™ Technology's effect on biofilm enhanced by ionic silver, and further by Ag+ Technology, which increases removal and disrupts the structure of the residual biofilm, improving the antimicrobial effect of the ionic silver ■ The synergistic action of ethylenediaminetetraacetate and benzethonium chloride disrupts the biofilm — with silver being bactericidal — to confer efficacy ***In vivo* data showed:** ■ Consistently decreased *P. aeruginosa* counts, and improved wound healing relative to inactive vehicle and active control wounds ($p < 0.05$)
2015[61,62]	**Real-life, clinical evaluations showed:** ■ Good wound closure rates, with indicated potential cost reductions ■ Reduced clinical infection signs and biofilm suspicion ■ Improved average treatment period, accompanied by high clinician satisfaction with efficacy and dressing change frequency
2016[63–65]	***In vitro* data showed** ■ The antimicrobial efficacy of ionic silver against biofilm is substantially improved by ionic silver with a metal chelating agent and a surfactant, which produce a synergistic effect (Ag+ Technology) **Real-life, clinical evaluations showed:** ■ Notable improvements in healing rates, ulcer condition, pain levels and wound area, with an acceptable safety profile ■ Wounds generally shifting from stagnant or deteriorating to improved, exudate levels improving, and tissue type moving from largely suspected biofilm to largely granulation tissue

Summary

Wound healing normally occurs in a predictable sequence, however, in some instances healing is prolonged or never achieved. The healing process is a complex interaction involving patient- and wound-related factors, the treatment used, and the skills and knowledge of healthcare professionals. Careful initial assessment and repeated evaluation of therapy are needed to recognise and assess the potential factors relating to wound complexity. In recent years, certain factors have been seen to have a considerable influence on healing, including wound infection, biofilm and exudate.

For healthcare professionals, initiating effective therapeutic strategies in a timely and cost-effective manner to reduce wound complexities, manage the patient's symptoms and expectations and, where possible, achieve healing, remains a challenge. Indeed, the drive towards securing funding for efficacious and cost-effective wound care therapies continues apace.

Innovative strategies for diagnosis and treatment are critical. Making changes in approach to wound care could lead to improved symptom control and long-term outcomes, reduced economic costs, and better patient quality of life. Exciting developments in the field of point-of-care diagnostic testing, which have been identified above, have the potential to facilitate improvements in practice and offer a more targeted and effective approach to wound management. The evolution of Hydrofiber™ Technology in dressings with the addition of anti-biofilm Ag+ Technology also presents the case for an innovative advanced technology for hard-to-heal wounds that combats certain factors with a considerable influence on healing: biofilm, exudate and risk of infection.

AUTHORS

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References

1. Kantor J, Margolis DJ. A multicentre study of percentage change in venous leg ulcer area as a prognostic index of healing at 24 weeks. *Br J Dermatol* 2000; 142(5):960–4.

2. Vowden P. *Hard-to-heal wounds Made Easy*. Wounds International 2011; 2(4): Available from http://www.woundsinternational.com

3. Acosta JB, del Barco DG, Vera DC, Savigne W, Lopez-Saura P, Guillen Nieto G et al. The pro-inflammatory environment in recalcitrant diabetic foot wounds. *Int Wound J* 2008; 5(4):530–9.

4. Phillips PL, Wolcott RD, Fletcher J, Schultz GS. *Biofilms Made Easy*. Wounds International 2010; 1(3): Available from http://www.woundsinternational.com

5. International consensus. *Making the case for cost-effective wound management*. Wounds international 2013. Available to download from www. woundsinternational.com

6. Dowsett C. Breaking the cycle of hard-to-heal wounds: balancing cost and care. *Wounds Int* 2015; 6 (2):17–21.

7. International consensus. *Optimising wellbeing in people living with a wound. An expert working group review*. London: Wounds International, 2012. Available from: www.woundsinternational.com

8. Evidence-Based Synthesis Programme (EBSP) (2010) *Advanced Wound Care Therapies for Non-Healing Diabetic, Venous, and Arterial Ulcers*: A Systematic Review. Available at: www.ncbi.nlm.nih.gov/pubmedhealth/PMH0054957/pdf/PubMedHealth_PMH0054957.pdf (accessed on 15.03.16)

9. James G, Swogger E, Wolcott R, Pulcini E, Secor P, et al. Biofilms in chronic wounds. *Wound Repair Regen* 2008 Jan 1; 16:37–44.

10. Percival S, Bowler P, 2004. Biofilms and their potential role in wound healing. *WOUNDS – A Compendium of Clinical Research and Practice* 2004 Jul 1; 16: 234–40.

11. Burmølle M, Thomsen TR, Fazli M, et al. Biofilms in chronic infections — a matter of opportunity — monospecies biofilms in multispecies infections. *FEMS Immunol Med Microbiol* 2010; 59:324–336.

12. Hurlow, J, Couch, K, Laforet, K, Bolton, L, Metcalf, D, Bowler, P, 2014. Clinical Biofilms: A Challenging Frontier in Wound Care. *Advances in wound care* 2015 May 1; 4:295–301.

13. Metcalf D, Bowler P. Biofilm delays wound healing: a review of the evidence. *Burns & Trauma* 2013; 1(1): 5–11.

14. Wolcott RD, Rhoads DD, Dowd SE. Biofilms and chronic wound inflammation. *J Wound Care* 2008; 17(8):333–41.

15. Leaper DJ, Schultz G, Carville K, Fletcher J, Swanson T and Drake R (2012). Extending the TIME concept: what have we learned in the past 10 years? *International Wound Journal* 9(Suppl 2): 1-19.

16. Bjarnsholt T (2013) The role of bacterial biofilms in chronic infections. *APMIS* 121 (Suppl 136): 1–51.

17. Läuchli S, Swanson T, Serena T, Harding K. The use of a point-of-care test for bacterial protease activity in chronic wounds. *Wounds International* 2015; 6(4).

18. Watters C, Everett J, Haley C, Clinton A, Rumbaugh K. Insulin treatment modulates the host immune system to enhance Pseudomonas aeruginosa wound biofilms. *Infect. Immun* 2014, 82(1):92-100.

19. Souza MC, dos Santos LS, Sousa LP, Faria YV, Ramos JN, Sabbadini PS, et al. Biofilm formation and fibrinogen and fibronectin binding activities by Corynebacterium pseudodiphtheriticum invasive strains. *Antonie van Leeuwenhoek*. 2015 Jun 1; 107(6):1387–99.

20. Sillanpaa J, Chang C, Singh KV, Montealegre MC, Nallapareddy SR, Harvey BR, et al. Contribution of individual Ebp Pilus subunits of Enterococcus faecalis OG1RF to pilus biogenesis, biofilm formation and urinary tract infection. *PloS one* 2013; 8(7):e68813.

21. Chavakis T, Wiechmann K, Preissner KT, Herrmann M. Staphylococcus aureus interactions with the endothelium: the role of bacterial 'secretable expanded repertoire adhesive molecules' (SERAM) in disturbing host defense systems. *Thrombosis and haemostasis* 2005; 94(2):278–85.

22. Singh R, Ray P. Quorum sensing-mediated regulation of staphylococcal virulence and antibiotic resistance. *Future microbiology* 2014; 9(5):669–81.

23. Laverty G, Gorman SP, Gilmore BF. Biomolecular mechanisms of staphylococcal biofilm formation. *Future microbiology* 2013; 8(4):509–24.

24. Coggan KA, Wolfgang MC. Global regulatory pathways and cross-talk control pseudomonas aeruginosa environmental lifestyle and virulence phenotype. *Current issues in molecular biology* 2012; 14(2):47–70.

25. Swanson T, Grothier L, Schultz G. *Wound Infection Made Easy*. Wounds International 2014. Available from: www.woundsinternational.com

26. Whitfield GB, Marmont LS, Howell PL. Enzymatic modifications of exopolysaccharides enhance bacterial persistence. *Front Microbiol* 2015; 6:471.

27. Moyat M, Velin D. Immune responses to infection. *WJG* 2014; 20(19):5583–93.

28. Durand E, Cambillau C, Cascales E, Journet L. VgrG, Tae, Tle, and beyond: the versatile arsenal of Type VI secretion effectors. *Trends in microbiology* 2014; 22(9):498–507.

29. Raymond B, Young JC, Pallett M, Endres RG, Clements A, Frankel G. Subversion of trafficking, apoptosis, and innate immunity by type III secretion system effectors. *Trends in microbiology* 2013; 21(8):430–41.

30. Torres VJ, Stauff DL, Pishchany G, Bezbradica JS, Gordy LE, Iturregui J, et al. A Staphylococcus aureus regulatory system that responds to host heme and modulates virulence. *Cell host & microbe* 2007; 1(2):109–19.

31. Harrison-Balestra C, Cazzaniga AL, Davis SC, Mertz PM. A wound-isolated Pseudomonas aeruginosa grows a biofilm in vitro within 10 hours and is visualized by light microscopy. *Dermatologic surgery* 2003; 29(6):631–5.

32. Schooling SR, Beveridge TJ. Membrane vesicles: an overlooked component of the matrices of biofilms. *Journal of bacteriology* 2006; 188(16):5945–57.

33. Diegelmann RF. Excessive neutrophils characterize chronic pressure ulcers. *Wound Repair Regen* 2003; 11(6):490–5.

34. Trengove NJ, Stacey MC, MacAuley S, Bennett N, Gibson J, Burslem F, et al. Analysis of the acute and chronic wound environments: the role of proteases and their inhibitors. *Wound Repair Regen* 1999; 7(6):442–52.

35. Mori S, Pawankar R, Ozu C, Nonaka M, Yagi T, Okubo K. Expression and Roles of MMP-2, MMP-9, MMP-13, TIMP-1, and TIMP-2 in Allergic Nasal Mucosa. *Allergy Asthma Immunol Res* 2012; 4(4):231–9.

36. Nwomeh BC, Liang HX, Cohen IK, Yager DR. MMP-8 is the predominant collagenase in healing wounds and non-healing ulcers. *The Journal of surgical research* 1999; 81(2):189–95.

37. Purevdorj-Gage B, Costerton WJ, Stoodley P. Phenotypic differentiation and seeding dispersal in non-mucoid and mucoid Pseudomonas aeruginosa biofilms. *Microbiology* 2005; 151(Pt 5):1569–76.

38. Hall-Stoodley L, Costerton JW, Stoodley P. Bacterial biofilms: from the natural environment to infectious diseases. *NatRevMicrobiol* 2004; 2(2):95–108.

39. Sun Y, Smith E, Wolcott R, Dowd SE. Propagation of anaerobic bacteria within an aerobic multi-species chronic wound biofilm model. *J Wound Care* 2009; 18(10):426–31.

40. Romanelli M, Vowden K, Weir D. Exudate management Made Easy. *Wounds Int* 2010; 1(2): available from http://www.woundsinternational.com

41. Bishop SM, et al. Moisture Balance: Optimizing the wound-dressing interface. *J of Wound Care* 2003; 12(4).

42. Minematsu T, Yamamoto Y, Nagase T, Nakagami G, Sugama J, Sanada H. Changes in tissue structure and barrier function in macerated skin. *J Jpn WOCM* 2011; 15: 278–281

43. Young T (2012) Using a protease test to inform wound care treatment decisions. *Wounds UK* 8(4): 74–80.

44. Dowsett C, Newton H. Wound bed preparation: TIME in practice. *Wounds UK* 2005 Mar; 1(3):58.

45. DaCosta RS, I Kulbatski, L Lindvere-Teene, D Starr, K Blackmore, J Silver, J Opoku, Y Wu, P Mededeiros, W Xu, L Xu, B Wilson, C Rosen, R Linden. Point-of-care autofluorescence imaging for real-time sampling and treatment guidance of bioburden in chronic wounds: First-in-human results. *PLoS one* Mar 2015 19; 10(3):e0116623.

46. Wu YC, M Smith, A Chu, L Lindvere-Teene, D Starr, K Tapang, R Shekhman, O Wong, R Linden, RS DaCosta. Handheld fluorescence imaging device detects subclinical wound infection in an asymptomatic patient with chronic diabetic foot ulcer: a case report. *Int Wound J* 2015 Apr 1.

47. Kanazawa T, Nakagami G, Goto T, Noguchi H, Oe M, Miyagaki T, Hayashi A, Sasaki S, Sanada H. Use of smartphone attached mobile thermography assessing subclinical inflammation: a pilot study. *J Wound Care* 2016; 25:177–82.

48. Tegl G, Schiffer D, Sigl E, Heinzle A, Guebitz GM. Biomarkers for infection: enzymes, microbes, and metabolites. *Appl Microbiol Biotechnol* 2015; 99:4595–614.

49. Thet NT, Alves DR, Bean JE, Booth S, Nzakizwanayo J, Young AE, Jones BV, Jenkins AT. Prototype Development of the Intelligent Hydrogel Wound Dressing and Its Efficacy in the Detection of Model Pathogenic Wound Biofilms. *ACS Appl Mater Interfaces* 2015, Oct 22 [Epub ahead of print].

50. Tamayol A, Akbari M, Zilberman Y, Comotto M, Lesha E, Serex L, Bagherifard S, Chen Y, Fu G, Ameri SK, Ruan W, Miller EL, Dokmeci MR, Sonkusale S, Khademhosseini A. Flexible pH-Sensing Hydrogel Fibers for Epidermal Applications. *Adv Healthc Mater* 2016 ; 5:711–9].

51. Hutchinson JJ and Lawrence JC . *Wound infection under occlusive dressings* 1991 Feb 28; 17(2):83–94.

52. Winter G. Formation of the Scab and the Rate of Epithelisation of Superficial Wounds in the Skin of the Young Domestic Pig. *Nature* 1962; 293–294.

53. Jones VJ. The use of gauze: Will it ever change? *I Wound* 2006; 3:79–86.

54. Thomas S. Hydrocolloid dressings in the management of acute wounds: a review of the literature. *Int Wound J* 2008; 5:602–13.

55. Queen D. Technology update: Understanding Hydrofiber® Technology. *Wounds International* 2010; 1(5).

56. Waring MJ, Parsons D. Physicochemical characterisation of carboxy-methylated spun cellulose fibres. *Biomaterials* 2000; 22: 903–12.

57. Walker M, Hobot JA, Newman GR, Bowler PG. Scanning electron microscopic examination of bacterial immobilisation in a carboxymethyl cellulose (AQUACEL®) and Alginate Dressing. *Biomaterials* 2003; 24: 883–90.

58. Parsons D. *Designing a dressing to address local barriers to wound healing. In: Next-generation antimicrobial dressings: AQUACEL™ Ag+ Extra™ and Ribbon*. London: Wounds International 2014 (Suppl). Available to download from: www.woundsinternational.com

59. Said J, Walker M, Parsons D, Stapleton P, Beezer AE, Gaisford S. An in vitro test of the efficacy of an anti-biofilm wound dressing. *Int J Pharm* 2014; 474:177–81.

60. Seth AK, Zhong A, Nguyen KT, Hong SJ, Leung KP, Galiano RD, Mustoe TA. Impact of a novel, antimicrobial dressing on in vivo, Pseudomonas aeruginosa wound biofilm: quantitative comparative analysis using a rabbit ear model. *Wound Repair Regen* 2014; 22:712–9.

61. Harding KG, Szczepkowski M, MikosiÐski J, Twardowska-Saucha K, Blair S, Ivins NM, Saucha W, Cains J, Peters K, Parsons D, Bowler P. Safety and performance evaluation of a next-generation antimicrobial dressing in patients with chronic venous leg ulcers. *Int Wound J* 2015. doi: 10.1111/iwj.12450.

62. Walker M, Metcalf D, Parsons D, Bowler P. A real-life clinical evaluation of a next-generation antimicrobial dressing on acute and chronic wounds. *J Wound Care* 2015; 24:11–22.

63. Metcalf D, Parsons D, Bowler P. A next-generation antimicrobial wound dressing: a real-life clinical evaluation in the UK and Ireland. *J Wound Care* 2016; 25:132–8.

64. Metcalf DG, Parsons P, Bowler PG. Clinical safety and effectiveness evaluation of a new antimicrobial wound dressing designed to manage exudate, infection and biofilm. *Int Wound J* 2016; doi: 10.1111/iwj.12590.

65. Bowler PG, Parsons D. Combatting wound biofilm and recalcitrance with a novel anti-biofilm Hydrofiber® wound dressing. *Wound Medicine* 2016; 14:6–11.