Acta Med Croatica, 70 (2016) 49-56 *Clinical Observation*

DEVELOPMENT OF A NEXT-GENERATION ANTIMICROBIAL WOUND DRESSING

DANIEL METCALF, DAVID PARSONS I PHILIP BOWLER

Science & Technology, ConvaTec Global Development Centre, Deeside, United Kingdom

Delayed wound healing due to infection is a burden on healthcare systems, and the patient and caregiver alike. An emerging factor in infection and delayed healing is the presence development of biofilm in wounds. Biofilm is communities of microorganisms, protected by an extracellular matrix of slime in the wound, which can tolerate host defences and applied antimicrobials such as antibiotics or antimicrobial dressings. A growing evidence base exists suggesting that biofilm exists in a majority of chronic wounds, and can be a precursor to infection while causing delayed healing itself. *In vivo* models have demonstrated that the inflammatory, granulation and epithelialization processes of normal wound healing are impaired by biofilm presence. The challenge in the development of a new antimicrobial wound dressing was to make standard antimicrobial agents more effective against biofilm, and this was answered following extensive biofilm research and testing. A combination of metal chelator, surfactant and pH control displayed highly synergistic anti-biofilm action with 1.2% ionic silver in a carboxymethylcellulose dressing. Its effectiveness was challenged and proven in complex in vitro and in vivo wound biofilm models, followed by clinical safety and performance demonstrations in a 42-patient study and 113 clinical evaluations. Post-market surveillance was conducted on the commercially available dressing, and in a 112-case evaluation, the dressing was shown to effectively manage exudate and suspected biofilm while shifting difficult-to-heal wounds onto healing trajectories, after an average of 4 weeks of new dressing use in otherwise standard wound care protocols. This was accompanied by a low frequency of dressing related adverse events. In a second evaluation, clinical signs of infection and wound dimension data, before and after the evaluations, were also available. Following an average of 5.4 weeks of dressing use, all signs of clinical infection were reduced, from an average frequency of 36% to 21%. An average of 62% wound size reduction was achieved, with 90% of wounds reducing in size and 10 wounds healing completely. The new clinical evidence for this next-generation antimicrobial wound dressing suggests it is safe and effective at managing exudate, infection and biofilm, while it can shift established, stubborn wounds onto healing trajectories. The scientific rationale for this new dressing technology is supported by *in vitro* and *in vivo* evidence, so now further comparative, randomized and outcome-based clinical studies are required to fully understand the clinical and economic benefits this new dressing technology can bring.

Key words: wound, biofilm, infection, antimicrobial dressing, anti-biofilm

Corresponding author: Daniel Metcalf, PhD
ConvaTec Global Development Centre
First Avenue, Deeside Industrial Park
Deeside, CH5 2NU, UK
Tel: +44 1244 584336
E-mail: daniel.metcalf@convatec.com

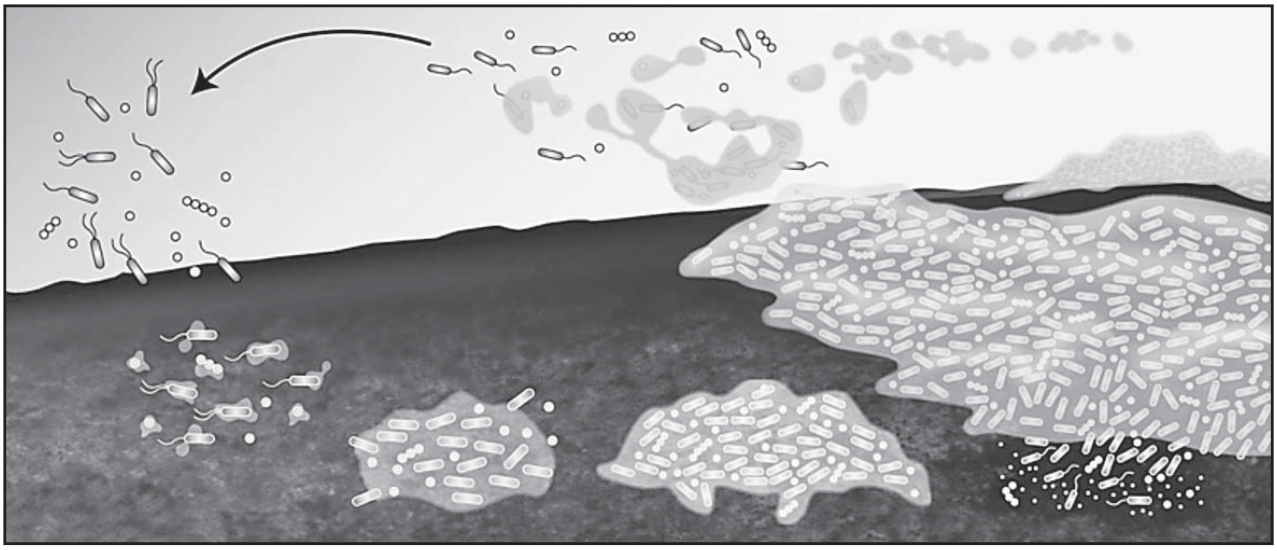
BACKGROUND

Delayed wound healing is a huge drain on healthcare budgets, and chronic wounds are estimated to account for 2-4% of global healthcare costs (1), or approximately €100-200 billion per year (2). The development of infection delays the normal wound healing process and results in increased burden on the patient and caregiver alike (3,4). Clinical infection develops when the body's innate immune defence mechanisms respond to the presence and invasion of pathogens, including endotoxin and exotoxin produced by the microorganisms themselves (5), via local inflammatory processes (6). These result in the familar, classic signs of wound infection – redness, heat, swelling, pain, odor, pus, as well as the more recently acknowledged addional signs, such as friable granulation tissue, discolouration, and delay to timely wound healing

(4). However, in the last 10-15 years, another factor in wound infection and delayed healing has emerged. The presence and development of microbial biofilm in wounds has been proposed (7,8), investigated (9), and then scientifically confirmed in chronic wounds (10,11).

Biofilm in wounds are communities of microrganisms adhered to each other and to wound surfaces, such as the wound bed, devitalised tissue, or dressings. The microorganisms are embedded in a matrix of extra-cellular polymeric substances (EPS), comparised mainly of polysaccharides, proteins and DNA, forming a complex, three-dimensional scaffold of slime in which they are protected (12). Biofilm microorganisms are thus physically protected in the slime, and also display slower growth rates and metabolic phenotypes than their planktonic counterparts (13). As a result, biofilm microorganisms can display tolerance towards systemic antibiotics and topically-applied antiseptics, such as silver or iodine (14), as well as host defence mechanisms, such as phagocytosis and enzyme-mediated microbial killing by neutrophils (15).

Figure 1 shows how planktonic, single-celled microorganisms, can attach to wound surfaces and, if not managed at this stage by an optimal host immune response or by antimicrobial agents, the microorganisms will secrete their sticky biofilm matrix. At this stage the biofilm will be microscopic but may start to illicit an inflammatory host response, and if unchecked, can develop into larger, more elaborate communities (16). At this stage, biofilm may be visible directly as slimy or milky substances on the wound bed, or indirectly *via* the signs of inflammation or infection which they illicit (17,18). Established wound biofilm therefore presents a serious infection risk, and may also seed biofilm formation elsewhere in the wound (19). As biofilm develops, so the risk of infection and delayed healing increases (Fig. 1).



Contamination ➡ Colonisation ➡ Biofilm development; ➡ Possible infection; Inflammatory host response Local ➡ spreading
Fig. 1. *Biofilm formation in wounds (originally published in (42).*

A growing body of scientific and clinical evidence now exists which suggests that wound biofilm, if not properly managed, may lead to wound infection and can delay healing (20). Dr Randall Wolcot, who practices biofilm-based wound care (BBWC) (21) in the US has stated that *"biofilms are the principle cause of wound chronicity"* (22). Whilst this statement has yet to be fully verified, the evidence that BBWC – the use of multiple, concurrent physical debridement and antimicrobial methods to target biofilm – can accelerate progression towards healing (23,24), with associated cost benefits (25), is growing. Scientific research within the last decade has confirmed that biofilm presence is correlated with wound chronicity, beginning in 2008, when two pioneering research groups in the US (10) and Denmark (11) independently observed biofilm microscopically in 60% of chronic wound samples.

Further powerful scientific evidence comes from *in vivo* animal models, for example, by the introduction of biofilm into carefully-created acute leporine ear wounds, which can then be monitored to evaluate the efficacy of anti-biofilm approaches (26). In this model, ineffective host inflammatory responses have been observed, such as failure of neutrophils to phagocytose (26) and kill pathogens *via* the oxidative burst (27), and well as differential inflammatory responses ellicited by different pathogenic biofilm species (28). The healing procesess

of granulation tissue formation and epithelialization have also been shown to be retarded by the presence of biofilm in the wound bed (26,28,29). Notably, using multiple concurrent strategies to target biofilm in this model has been shown to be more effective at encouraging wound healing than individual techniques (30). The emerging problem of wound biofilm presents a significant challenge for developers of new, more effective wound dressings. The aim of this review is to summarize the development of a new dressing technology designed to be effective against wound biofilm.

DESIGNING A NEW ANTI-BIOFILM TECHNOLOGY

Formulation

The market-leading antimicrobial wound dressing, a nonwoven carboxymethylcelluose (CMC) dressing containing 1.2% ionic silver (AQUACEL™ Ag), has been on the market since 2002, before the 'biofilm era' of wound care really began (8-11). Although this dressing shows some effectiveness against biofilm in laboratory models (31,32), its silver ions, unaided, are slow to reach the microorganisms protected within the biofilm. When considering how to make ionic silver far more effective against biofilm, simply adding higher concentrations of silver would have safety implications (33) and would alter the physical attributes of the CMC dressing (exudate absorption and retention, gelling, conformability to the wound bed, etc.) (34). A comprehensive research and development program firstly focused on understanding the general structure, composition and behaviour of wound biofilm. Knowing that the complex three-dimensional matrix of EPS was generally comprised of hydrated polysaccharides, protein and DNA of microbial origin, as well as host-derived devitalised tissues (12,35), the search for safe and effective means by which to target this matrix was undertaken. The hypothesis was that if the biofilm matrix could be destabilized and the microorganisms within be exposed, then the antimicrobial activity of the silver CMC base dressing would be maximized.

Several modes of action of biofilm destabilization were theorized, such as enzymatic action, solubilization, ion exchange, metal chelation, ionic and nonionic surfactancy, and pH control. Following tens of thousands of laboratory tests using a well-characterized rapid screening assay for anti-biofilm activity (36), an optimum combination of low levels of metal chelator and a surfactant, combined with pH control was identified. When formulated with 1.2% ionic silver, this combination demonstrated highly synergistic anti-biofilm activity. The metal chelator was selected due to its ability to sequester divalent metal ions, such as Fe^{2+}, Ca^{2+} and Mg^{2+}, which are known to hold the polymers of the biofilm matrix scaffold together (37,38). The surfactant selected has a detergent-like action which further loosens the biofilm, reducing surface tensions within the dressing and biofilm, allowing the ionic silver to move more quickly (39). Finally, an acidic pH of 5.5 was identified as optimum in order for the other new components and ionic silver to function most effectively (40,41). Notably, the CMC remained the same, and the level of silver remained the same as in the original silver CMC dressing (1.2%).

EVIDENCE FOR A NEW ANTI-BIOFILM TECHNOLOGY

Anti-biofilm effectiveness testing

This unique formulation of anti-biofilm excipients and ionic silver was named '*Ag+ Technology*', and its effectiveness was verified following manufacture into the CMC dressing form (CMC-Ag+) using a complex *in vitro* gauze biofilm model. Briefly, mature community-associated Methicillin-resistant *Staphylococcus aureus* (CA-MRSA) or *Pseudomonas aeruginosa* biofilm was grown on gauze dressing pieces, before being transferred to agar plates (simulating the wound bed) mounted in a leather surround (simulating the peri-wound skin). The dressing was then applied to the biofilm, a secondary dressing applied, and its anti-biofilm performance assayed at frequent time points by viable counting and microscopic image analysis. Compared to the base silver CMC dressing (42), and two other commercially-available silver dressings containing 2.2% and 11.2% silver (43), the CMC-Ag+ dressing showed significantly superior effectiveness against CA-MRSA and *P. aeruginosa* biofilm, in terms of bacterial killing and prevention of biofilm re-reformation following re-inoculation (Table 1). This effectiveness was subsequently confirmed in an independent model that used microcalorimetry to measure anti-biofilm activity via changes in total biofilm metabolism (44) (Table 1).

The safety and performance of the CMC-Ag+ dressing was evaluated in external toxicity and performance studies, as required by regulatory authorities, including in a well-characterized *in vivo* model (26). Briefly, *P. aeruginosa* biofilm, or *P. aeruginosa* and *S. aureus* polymicrobial biofilm, was allowed to develop in acute wounds of defined size, and the effect of the applied CMC-Ag+ was measured by viable counts and the wound closure parameters of granulation tissue formation and epithelialization. The dressing was found to be significantly superior to a polyhexanide-containing antimicrobial dressing in reducing biofilm counts and achieving wound healing progression (45) (Table 1).

Table 1.
Summary of initial in vitro, in vivo and clinical studies for the CMC-Ag+ dressing (42-47). 1: AQUACEL® Ag (ConvaTec); 2: Silvercel™ Non Adherent (Systagenix, UK); 3: Acticoat™ 7 (Smith & Nephew, UK); 4: AQUACEL® (ConvaTec); 5: Telfa™ AMD™ (Covidien, US).

Study type	Model	Comparators	Results	Reference
In vitro	Gauze biofilm model; viable biofilm counts	Silver (1.2%) CMC dressing[1]	CMC-Ag+ dressing gave 6 log_{10} kill of *P. aeruginosa* and CA-MRSA biofilm after 4 and 5 days; standard silver CMC did not fully eradicate either biofilm	42
In vitro	Gauze biofilm model; viable biofilm counts	(i) Silver (2.2%) alginate-CMC-nylon dressing[2]; (ii) nanocrystalline silver (11.2%) rayon-polyester-polyethylene dressing[3]	Silver alginate-CMC-nylon dressing and nanocrystalline silver dressing did not reduce P. aeruginosa or CA-MRSA biofilm counts	43
In vitro	Agar-supported biofilm; isothermal microcalorimetry	(i) CMC dressing[4]; (ii) silver (1.2%) CMC dressing[1]; (ii) silver nitrate solution (0.1 M); (iv) ethylenediaminotetraacetic acid (EDTA; metal chelator); (v) benzethonium chloride (BC; surfactant)	CMC-Ag+ dressing and silver nitrate+EDTA+BC eradicated *S. aureus* biofilm; silver CMC dressing, CMC dressing, EDTA+BC, and silver nitrate alone did not eradicate the biofilm; demonstrating synergy of silver with metal chelator and surfactant	44
In vivo		(i) Polyhexanide gauze dressing[5]; (ii) CMC dressing[4]	CMC-Ag+ dressing gave 2 log_{10} reductions in *P. aeruginosa* biofilm after 4 and 6 days compared to polyhexanide gauze and CMC dressings; granulation tissue formation and epithelialization was significantly better in CMC-Ag+ dressed wounds; similar improvements in polymicrobial wounds	45
Clinical study	Clinical safety & performance in venous leg ulclers	None	Acceptable safety profile demonstrated; after 4 weeks CMC-Ag+ dressing then 4 weeks CMC 12% of wounds healed, 76% showed improvement in wound health; mean ulcer size reduction was 55%; subset of 10 infected wounds reduced in area by 70%	46
Clinical evaluations	Evaluations in chronic and acute wounds	None (previously used primary dressings were recorded)	CMC-Ag+ dressing resulted in an average wound closure of 73% after average of 4.1 weeks; 17% of wounds healed	47

Clinical assessment

The CMC-Ag+ dressing was also evaluated clinically in two separate investigations. The first was primarily a clinical safety study involving 42 patients with venous leg ulcers, of which 10 were deemed to be clinically infected. Whilst safety and progress towards wound closure was demonstrated for all wounds, in particular the 10 infected wounds, where biofilm was likely a factor, responded in more dramatic fashion, following 4 weeks of management with the CMC-Ag+ dressing then 4 weeks with the base CMC dressing (46; Table 1). Clinical evaluations on this now CE-Marked dressing were also conducted across Europe and Canada, where 113 hard-to-heal wounds were selected based on suspicion of infection (4) or biofilm (17) based on clinical signs. After an average of 4.1 weeks use of the CMC-Ag+ dressing in otherwise standard care protocols, 73% average wound closure was achieved, with 17% of wounds progressing to complete healing (47) (Table 1).

New clinical evidence

The version of this dressing designed to manage wound biofilm that is now commercially-available in Europe and Canada since 2014, AQUACEL® Ag+ EXTRA™ (CMC-Ag+E), is thicker to give improved absorption and also contains strengthening yarn to aid complete dressing removal (42). Since its launch, post-market surveillance evaluations have been conducted in the United Kingdom to further investigate the safety and effectiveness of the dressing. Clinicians in healthcare facility and community settings across the UK were invited to select challenging cases of hard-to-heal wounds that were stalled or deteriorating, and that might be compromised by infection (4) or suspected biofilm (17). Clinicians were asked to consider switching the previously used primary antimicrobial dressing to CMC-Ag+E for to 4 weeks, or as long as they considered it appropriate. All other standard local protocols were followed, and the clinicians recorded patient and various wound data throughout the evaluations on

simple forms. Two data sets emerged; the first was a 112-case population where safety and effectivess could be examined using baseline and post-evaluation data on exudate, wound bed appearance (including suspected biofilm), wound progression, and dressing-related adverse events. A smaller data set of 29 cases where pre- and post-evalution data on signs of infection and wound dimensions were also available.

Safety & effectiveness

Baseline and post-evaluation data from the 112-case clinical evaluation is summarized in Table 2. Most of the wounds were stagnant with over a quarter classed as deteriorating at baseline. Almost one-third were judged to be infected, and biofilm suspicion of 54% was greater than any other individual clinical sign of infection (data not shown). Duration of use of the CMC-Ag+E dressing was less than 4 weeks on average. Exudate levels, which were previously high or medium, were shifted to low or medium. Biofilm suspicion, as judged by visible or indirect signs (17), was more than halved. In terms of the wound bed appearance, before the evaluations, the main tissue type observed was suspected biofilm or slough. After use of the CMC-Ag+E dressing, this suspected biofilm and slough was reduced, while granulation tissue had become the main wound bed tissue type. Notably, these stalled and deteriorating wounds were shifted onto healing trajectories, with a majority of the wounds improving and 14 healing completely. Perhaps most importantly, there were only a small number of dressing-related adverse events. Only 3 such events were identified, and 2 of these were mild stinging which resolved quickly. There was one unexplained 'reaction' to the dressing, but overall, the CMC-Ag+E dressing was well tolerated and appeared to have an acceptable safety profile, as had been previously determined (46).

Table 2.
Summary of new clinical evidence for the CMC-Ag+E dressing.

	Data set 1	**Data set 2** (48)
Baseline data		
Number of wounds	112 (111 patients)	29 (28 patients)
Median/mean duration	12 months/32 months	10 months/34 months
Wound status Stagnant Deteriorating	65% 27%	72% 24%
Classed as clinically infected	31%	24%
Biofilm suspicion (yes/no judgment)	54%	76%
Results		
Mean dressing use duration	3.9 weeks	5.4 weeks
Exudate rating Before After	54% medium, 43% high 48% low, 44% medium	55% medium, 34% high 41% medium, 34% low
Main wound bed tissue Before types (% judgment) After	40% suspected biofilm, 31% slough 46% granulation tissue, 17% slough	49% suspected biofilm, 27% slough 53% granulation tissue, 24% biofilm
Biofilm coverage (% judgment) Before After	40% 16%	N/A N/A
Signs of infection (+ biofilm) Before (mean frequency of 9 signs) After	N/A N/A	36% 21%
Wound closure	65% improved, 13% healed	90% improved, 34% healed, 62% average wound closure
Adverse events	3 (2 mild stinging, 1 unknown 'reaction')	N/A

Infection and wound closure

Baseline and post-evaluation data from the 29 cases where signs of infection and wound closure data was also available is summarized in Table 2 (48). Baseline data was generally similar to the larger study, with a majority of wounds stagnant and approximately one-quarter deteriorating, although clinical infection classification was slightly lower at one-quarter of wounds, while biofilm suspicion higher at 76%. Duration of dressing usage was 5.4 weeks on average, and as in the larger study, exudate levels were reduced after the evaluations, and wound bed tissue types were shifted from suspected biofilm and slough to mainly granulation tissue. In addition to suspected biofilm, the frequency of 8 clinical signs of infection were recorded (erythema, oedema, heat/warmth, pain, odour, excessive exudate, and discolured or friable granulation tissue) (4). For each of these signs, frequency was reduced following the dressing evaluations, with the

average frequency reducing from 36% to 21%. Wound closure data available for these 29 cases shows that 10 wounds completely healed and 90% reduced in size, with an average closure of 62% achieved. The wounds that fully healed were, on average, 14 months in duration yet it took just 6.7 weeks on average to heal these wounds using the CMC-Ag+E dressing (48). Upon investigation of 3 wounds which did not get smaller, it was found that 2 were from the same patient who was being administered systemic antibiotics, and the other was a long-standing wound of 7 years in a patient with peripheral arterial disease. This new clinical data on the effectiveness of this next-generation antimicrobial wound dressing to not only manage exudate, infection and biofilm, but to also shift challenging wounds onto healing trajectories, provides further evidence for this specifically developed new anti-biofilm technology.

CONCLUSION

The development of a next-generation antimicrobial wound dressing, based on the new field of wound biofilmology, has been shown to be effective in a number of *in vitro*, *in vivo* and clinical studies/evaluations. The dressing combines effective anti-biofilm action, enabling the activity of the well-established, safe antimicrobial agent, ionic silver, with a moist wound healing environment provided by the CMC dressing base. Emerging clinical evidence suggests that this dressing is able to shift challenging wounds onto healing trajectories where other antimicrobial therapies have not. This early promise should be investigated to understand the potential costs savings – wound care products, antibiotics (49), clinician time, clinical stay costs, etc. (50) – associated with the use of a dressing which progresses chronic wounds towards healing or resolves infected wounds. Comparative, randomized and outcome-based studies may provide even stronger clinical evidence for this new dressing technology.

REFERENCES

1. Gottrup F, Apelqvist J, Bjansholt T *et al*. EWMA Document: Antimicrobials and Non-healing Wounds—Evidence, Controversies and Suggestions. J Wound Care 2013; 22 (5 Suppl): S1-S92.

2. International Diabetes Federation Diabetes Atlas, Seventh edition, 2015. Available at: http://www.diabetesatlas.org/ (Accessed on 1st February 2016).

3. Loewenthal J. Sources and Sequelae of Surgical Sepsis. BMJ 1962; 1: 1437-40.

4. Cutting KF, Harding KG. Criteria for identifying wound infection. J Wound Care 1994; 3: 198-201.

5. Ovington L. Bacterial toxins and wound healing. Ostomy Wound Manage 2003; 49(7A Suppl): 8-12.

6. European Wound Management Association (EWMA). Position Document: Identifying criteria for wound infection. London: MEP Ltd, 2005.

7. Bowler PG. 1999. The Prevalence and Significance of Anaerobic Bacteria in Wounds. MPhil Thesis; University of Wales College of Medicine.

8. Percival SL, Bowler PG. Biofilms and their potential role in wound healing. Wounds 2004; 16:234-40.

9. Serralta VW, Harrison-Balestra C, Cazzaniga AL, Davis SC, Mertz M. Lifestyles of bacteria in wounds: Presence of biofilms? Wounds 2001; 13: 29-34.

10. James GA, Swogger E, Wolcott R et al. Biofilms in chronic wounds. Wound Repair Regen 2008; 16: 37-44.

11. Kirketerp-Møller K, Jensen PØ, Fazli M *et al.* Distribution, organization, and ecology of bacteria in chronic wounds. J Clin Microbiol 2008; 46: 2712-22.

12. Flemming H-C, Wingender J. The biofilm matrix. Nat Rev Microbiol 2010; 8: 623-33.

13. Percival SL, Hill KE, Malic S *et al*. Antimicrobial tolerance and the significance of persister cells in recalcitrant chronic wound biofilms. Wound Repair Regen 2011; 19: 1-9.

14. Bjarnsholt T, Kirketerp-Møller K, Jensen PØ *et al.* Why chronic wounds will not heal: a novel hypothesis. Wound Repair Regen 2008; 16: 2-10.

15. Edwards R, Harding KG. Bacteria and wound healing. Curr Opin Infect Dis 2004; 17: 91-6.

16. Mah TF, O'Toole GA. Mechanisms of biofilm resistance to antimicrobial agents. Trends Microbiol 2001; 9: 34-9.

17. Metcalf DG, Bowler PG, Hurlow J. A clinical algorithm for wound biofilm identification. J Wound Care 2014; 23: 137-142.

18. Metcalf DG, Bowler PG. Clinician perceptions of wound biofilm. Int Wound J 2014; doi: 10.1111/iwj.12358.

19. O'Toole G, Kaplan HB, Kolter R. Biofilm formation as microbial development. Annu Rev Microbiol 2000; 54: 49-79.

20. Metcalf DG, Bowler PG. Biofilm delays wound healing: a review of the evidence. Burns Trauma 2013; 1: 5-12.

21. Wolcott RD, Rhoads DD. A study of biofilm-based wound management in subjects with critical limb ischaemia. J Wound Care 2008; 17: 14555.

22. Wolcott RD, Rhoads DD, Bennett ME, Wolcott BM, Gogokhia L, Costerton JW, Dowd SE. Chronic wounds and the medical biofilm paradigm. J Wound Care 2010; 19: 45-53.

23. Wolcott RD, Kennedy JP, Dowd SE. Regular debridement is the main tool for maintaining a healthy wound bed in most chronic wounds. J Wound Care 2009; 18: 54-56.

24. Wolcott RD. Disrupting the biofilm matrix improves wound healing outcomes. J Wound Care 2015; 24: 366-71.

25. Wolcott R. Economic aspects of biofilm-based wound care in diabetic foot ulcers. J Wound Care 2015; 24: 189-94.

26. Gurjala AN, Geringer MR, Seth AK *et al*. Development of a novel, highly quantitative in vivo model for the study of biofilm-impaired cutaneous wound healing. Wound Repair Regen 2011; 9: 400-10.

27. Nguyen KT, Seth AK, Hong SJ *et al*. Deficient cytokine expression and neutrophil oxidative burst contribute to impaired cutaneous wound healing in diabetic, biofilm-containing chronic wounds. Wound Repair Regen 2013; 21: 833-41.

28. Seth AK, Geringer MR, Galiano RD *et al*. Quantitative comparison and analysis of species-specific wound biofilm virulence using an *in vivo*, rabbit-ear model. J Am Coll Surg 2012; 215 : 388-99.

29. Seth AK, Geringer MR, Hong SJ *et al*. Comparative analysis of single-species and polybacterial wound biofilms using a quantitative, *in vivo*, rabbit ear model. PLoS One 2012; 7: e42897.

30. Seth AK, Geringer MR, Gurjala AN *et al*. Treatment of *Pseudomonas aeruginosa* Biofilm–Infected Wounds with Clinical Wound Care Strategies: A Quantitative Study Using an In Vivo Rabbit Ear Model. Plast Reconstr Surg 2012; 129: 262e.

31. Percival SL, Bowler P, Woods EJ. Assessing the effect of an antimicrobial wound dressing on biofilms. Wound Repair Regen 2008; 16: 52-7.

32. Percival SL, Bowler PG, Dolman J. Antimicrobial activity of silver-containing dressings on wound microorganisms using an *in vitro* biofilm model. Int Wound J 2007; 4: 186-91.

33. Lansdown ABG. Uptake and metabolism of silver in the human body. In: Silver in healthcare: its antimicrobial efficacy and safety in use. Issues in Toxicology No 6. Cambridge: Royal Society of Chemistry, 2010, 43-71.

34. Williams C. An investigation of the benefits of Aquacel Hydrofibre wound dressing. Br J Nurs 1999; 8: 676-80.

35. Sutherland IW. The biofilm matrix – an immobilized but dynamic microbial environment. Trends Microbiol 2011; 9: 222-7.

36. Ceri H, Olson ME, Stremick C *et al*. The Calgary Biofilm Device: New technology for rapid determination of antibiotic susceptibilities in bacterial biofilms. J Clin Microbiol 1999; 37: 1771-6.

37. Rowe RC, Sheskey PJ, Weller PJ, eds. Handbook of Pharmaceutical Excipients, 4th edition. The Pharmaceutical Press, 2003.

38. Kostakioti M, Hadjifrangiskou M, Hultgren SJ. Bacterial biofilms: development, dispersal, and therapeutic strategies in the dawn of the postantibiotic era. Cold Spring Harb Perspect Med 2013; 3: a010306.

39. Pharmaceutics: Basic Principles and Application to Pharmacy Practice. Dash AK, Singh S, Tolman J. Academic Press, 2014.

40. Slone W, Linton S, Okel T *et al*. The effect of pH on the antimicrobial efficiency of silver alginate on chronic wound isolates. J Am Coll CWS 2010; 2: 86-90.

41. Jones EM, Cochrane CA, Percival SL. The Effect of pH on the Extracellular Matrix and Biofilms. Adv Wound Care (New Rochelle) 2015; 4: 431-9.

42. 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.

43. Parsons D, Bowler P. Design of a Next Generation Antimicrobial Hydrofiber® Dressing to Combat Wound Biofilm. Manuscript in preparation.

44. Said J, Walker M, Parsons D *et al*. An *in vitro* test of the efficacy of an anti-biofilm wound dressing. Int J Pharm 2014; 474: 177-81.

45. Seth AK, Zhong A, Nguyen KT *et al*. 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.

46. Harding, KG, Szczepkowski M, Mikosiński J *et al*. 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.

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

48. Metcalf D, Parsons D, Bowler P. A Real-Life Clinical Evaluation of a New Next-Generation Antimicrobial Wound Dressing in the United Kingdom. J Wound Care 2016; in press.

49.Gürgen M. Excess use of antibiotics in patients with non-healing ulcers. EWMA J 2014; 14: 17-22.

50. Harding K, Posnett J, Vowden K. A new methodology for costing wound care. Int Wound J 2012; 10: 623-9.

SAŽETAK

RAZVOJ NAJNOVIJE GENERACIJE ANTIMIKROBNE OBLOGE

D. METCALF, D. PARSONS i P. BOWLER

Znanost i tehnologija, Razvojni centar ConvaTec Global, Deeside, Ujedinjeno Kraljevstvo

Odgođeno cijeljenje rana zbog infekcije je teret zdravstvenim sustavima, a jednako tako i pacijentu i onome koji skrbi za njega. Bitan čimbenik u nastajanju infekcije i odgođenog cijeljenja je razvoj i prisutnost biofilma u ranama. Biofilm je zajednica mikroorganizama, zaštićena izvanstaničnim sluzavim matriksom u rani, koji može tolerirati obranu domaćina i primijenjena antimikrobna sredstva, kao što su antibiotici ili antimikrobne obloge. Rastući broj znanstvenih dokaza upućuje da biofilm već egzistira u većini kroničnih rana, a može biti i prethodnik infekciji dok istodobno uzrokuje odgođeno cijeljenje. *In vivo* modeli pokazali su da su upala, granulacija i epitelizacija, te procesi normalnog cijeljenja rane narušeni prisustvom biofilma. Izazov u razvoju nove antimikrobne obloge za ranu bio je da standardna antimikrobna sredstva učinimo učinkovitija protiv biofilma, a rješenje je uslijedilo nakon opsežnih istraživanja i ispitivanja biofilma. Kombinacija metalnog kelatora, površinski aktivne tvari i kontrole pH faktora pokazala je snažnu sinergističku anti-biofilm akciju u oblozi od karboksimetilceluloze sa 1,2 % ionskog srebra. Ta je učinkovitost testirana i dokazana u kompleksnim *in vitro* i *in vivo* modelima rana s biofilmom, a zatim i u klinički kontroliranim studijama, i to u studiji na 42-pacijenta i 113 kliničkih evaluacija. Naknadno ispitivanje nastavljeno je nakon dostupnosti obloge u evaluaciji na 112 slučaja, gdje je obloga pokazala učinkovito kontroliranje eksudata i suspektnog biofilma na ranama koje teško cijele i pri tome poticanje procesa cijeljenja rana i to nakon prosječno 4 tjedna primjene nove obloge u inače standardnom protokolu njege. To je bilo popraćeno niskim brojem nuspojava. U drugoj procjeni bili su evaluirani i klinički znakovi infekcije i podatci o veličini rane, prije i nakon procjene. Nakon prosječno 5,4 tjedana uporabe obloge, svi su klinički znakovi infekcije bili reducirani, s prosječnom učestalošću od 36 % do 21 %. U prosjeku u 62 % rana postignuta je redukcija veličine, uz smanjenje veličine do 90 % i 10 potpuno zacijeljenih rana. Najnoviji kliničkih dokazi za novu generaciju antimikrobne obloge za ranu potvrđuju njenu sigurnost i učinkovitost u kontroli eksudata, infekcije i biofilma, a osim toga potvrđuju i zacijeljivanje rana koje dugo i teško ili uopće ne cijele. Znanstvenu potporu za najnoviju tehnologiju i generaciju antimikrobne obloge potvrđuju *in vitro* i *in vivo* dokazi, tako da su buduća komparativna i randomizirana klinička ispitivanja neophodna za potpuno razumijevanje kliničke i ekonomske učinkovitosti koju može donijeti ova najnovija tehnologija.

Ključne riječi: rana, biofilm, infekcija, antimikrobna obloga, anti-biofilm