Intelligent Wound Dressing for Wound Infection Management and Diagnosis at Point of Care

N. T. Thet,1 D. R. Alves,2-4 J. E. Bean,1 S. Booth,4 J. Nzakizwanayo2, A. E. R. Young,5 B. V. Jones,2,4 A. T. A. Jenkins1

1Department of Chemistry, University of Bath, BA2 7AY, UK
2School of Pharmacy and Biomolecular Sciences, University of Brighton, BN2 4JG, UK
3Blond McIndoe Research Foundation, West Grinstead, West Sussex, RH19 3DZ, UK
4Queen Victoria Hospital, East Grinstead, West Sussex, RH19 3DZ, UK
5Healing Foundation Children’s Burns Research Centre, Bristol, BS2 8BJ, UK

Abstract
Here we present the development of a prototype wound dressing that can detect the infection in wounds. The dressing is made of a hydrated agarose sheet in which a mixture of fluorescent dye containing vesicles and agarose are dispersed within the hydrogel matrix. The release of dye is triggered by the interaction of vesicles with virulence factors, secreted within the biofilms via population-density-dependent quorum sensing, from clinical pathogens of Staphylococcus aureus, Pseudomonas aeruginosa and Enterobacter faecalis (SPE pathogen group). The dressing only activates when in contact with wound biofilm of pathogenic bacteria in infected wounds, but not to the biofilms of non-pathogenic bacteria.

Introduction
Wound infection is a global problem and approximately 13,000 patients with burns require treatment in hospitals in England and Wales every year.1,2 Burn wound infection is currently diagnosed by clinical observation and judgement, and standard microbiological culture to identify causative pathogens usually take several days.3,4 There are evidences that the delayed healing due to persistent infection is closely related to wound biofilm formation.5,6 If pathogens present, this will cause tissue damage by further colonization, extensive infection and formation of difficult-to-treat biofilm in wounds that inevitably require aggressive antibiotic treatments.7 Early indication of infection at point of care and ability to rapidly distinguish between infected and non-infected states of wound will help in clinical decision making, prevent over-management by inappropriate use of antibiotics, improve patient outcomes and reduce costs of treatment.

Methods, Results and Discussion

1) Vesicles and Mode of Action Detection
Vesicles of 200 nm in diameter were made of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), cholesterol and 10,12-tricosanoylacyl glycerol (TCDA) by extrusion.4 Encapsulated inside vesicles were 50 mM dim-3,5,6-carboxyfluorescein (CF) (regions of pathogenic bacteria, such as Pseudomonol-Soluble Modulin (PSM), delta haemolysin and rhamnolipids lysed vesicles and triggered the release of visible dye (figure 1).3,4) Expression of such virulence factors as S. aureus, P. aeruginosa was demonstrated by growing them on the blood agar plate (figure 2).

2) Dressing Design and Prototype Development
The vesicles were mixed with wet 0.7% agarose and filled inside the patterned wouleds of moulded 2% agarose dressing (figure 3a,b).2) This design allows the diffusion of toxins from infected wound to the dressing, following the fluorescent dyes release from vesicles within hydrogel matrices (figure 3c).

3) Clinical Assessment of Prevalent Burn Wound Pathogens
An independent assessment of S5 burn wounds at Queen Victoria Hospital in East Grinstead, UK provided that more than 75% of “at risk” wounds were colonized by species of pathogenic bacteria, of which above 80% were identified as S. aureus, P. aeruginosa and E. faecalis strains (SPE pathogen) (figure 4).

4) In-vitro Colony Wound Biofilm Model
For the evaluation on prototype dressing performance, a model wound biofilm was developed by growing biofilms on a nano-porous polycarbonate membrane (200 nm in diameter) on top of nutrient agar, incubated at 37°C (figure 5a,b).1,2 Growth of biofilm was characterized by Scanning Electron Microscope (figure 5c).

5) Dressing Response to In-vitro Model Wound Biofilms
In-vitro colony wound biofilms, produced from burn pathogens, S. aureus and P. aeruginosa, and control E. coli were grown for 24, 48 and 72 hours and tested with prototype dressings (figure 6a). Within 5 hours of incubation with biofilms, the fluorescent response was clearly observed in dressings (figure 6b).

6) Ex-vivo Porcine Burn Wound Model and Dressing Response
Using organic porcine skin, ex-vivo burn wound, as a more realistic model, was further developed for the evaluation of the dressing performance.5,6 Scaled burns were created with a hot metal block on disinsected pig skins (figure 7a) and infected with selected strains of SPE pathogens to establish biofilms allowing only burned tissues as a sole carbon source for infected bacteria (figure 7b). After 24 hours of growth at 37°C, each dressing was placed on the burn wounds and incubated up to 24 hours to test the dressing response (figure 7c). Fluorescent response was observed in all of the dressings except for the control dressings of E. coli.

Conclusion
A prototype intelligent wound dressing is developed and the dressing performance is accessed by testing with in-vitro and ex-vivo porcine wound biofilm models of clinically important S. aureus, P. aeruginosa and E. faecalis (SPE) pathogens. Patterned vesicles in hydrogel dressing are lysed by virulence factors of pathogenic bacteria in moved biofilms only and triggered the release of fluorescent dyes which are visible to naked eyes. This study provides proof-of-concept for an advanced infection-detecting dressing for wound care, which could allow the targeted treatment of infections at the bedside and reduce the unnecessary use of antibiotics.

Acknowledgement
The authors express the gratitude to Dr Jonathan Caplin (Univ. Brighton) for providing clinical strains of E. faecalis, E. coli and S. aureus for supporting valuable data for a study survey on burn wounds SPE pathogens. The authors also gratefully acknowledge the financial support from the Medical Research Council through “DPPC, EPSRC Health & Wellbeing Programme Grant: EP/K021068/1 (the European Commission’s Framework program FP7 project no. 245500 Bacteriostel, the Healing Foundation for support via the Children’s Burns Research Center, the James Tait Foundation for support of research nurse B.H.C., and the Annie Charitable Trust for PhD student support.

References

Figure 1. Schematic depiction of mode of action of vesicles against pathogenic bacteria. (a) Pathogens produce toxins that can lyse lipid bilayer membrane of vesicles, and (b) trigger the release of vesicles from vesicles within the hydrogel matrix.

Figure 2. Vesicles used to release CF-3,5,6-carboxyfluorescein (CF) inside vesicles, which were mixed with 0.7% agarose and filled inside the patterned moulds of 2% agarose dressing.

Figure 3. Prototype dressing design. (a) Embossed mold is used to make a thin sheet of agarose with patterned wells, which are filled with vesicles + agarose, (b) finished prototype, and (c) finished dressing after the injection of dressing with Tris on an arm.

Figure 4. Clinical assessment of prevalent burn wound pathogen. (a) 75% of infected wounds are colonized by pathogenic bacteria while 6% of them are identified as S. aureus, P. aeruginosa and E. faecalis strains.

Figure 5. In-vitro colony wound biofilm model (a) schematic depiction of growth of biofilm by acquiring nutrients through nanotubular structures of EPS matrix, (b) photograph of dressings with biofilm taken under UV (254 nm) light, and (c) growth of P. aeruginosa, S. aureus and E. coli.

Figure 6. Fluorescent response of prototype dressing in in-vitro model wound biofilm (a) time dependent response of dressing with 24 hour biofilms, and (b) photograph of dressings with biofilm taken under UV (254 nm) light.

Figure 7. Ex-vivo porcine burn wound model and fluorescent response of prototype dressing (a) scaled burn on pig skin, (b) 24 hour biofilm of P. aeruginosa, and (c) dressing response to porcine wound biofilms, seen under UV (254 nm) light.