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

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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 by population-density-dependent quorum sensing, from clinical pathogens of Staphylococcus aureus, Pseudomonas aeruginosa and Enterococcus 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 delay to the 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 biofilms in wounds which 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-tetradecanoylphosphatidyethanolamine (TCDA) by extrusion.8 Encapsulated inside vesicles were 50 mmol dm⁻³ self-quenched 5,6-carboxyfluorescein diacetate (HEPES) buffer. Only exotoxins of pathogenic bacteria, such as S. aureus, P. aeruginosa and E. faecalis were demonstrated by showing them on the blood agar plate (figure 2).

2) Dressing Design and Prototype Development
The vesicles were mixed with molten 0.7% agarose and filled into the patterned wells of moulded 2% agarose dressing (figure 3a,b).5 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 55 burn wounds at Queen Victoria Hospital (QVH) at East Grinstead, U.K. 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 pathogens) (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). 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 skins, ex-vivo burn wound, as a more realistic model, was further developed for the evaluation of the dressing performance.11 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 biofilms 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 mixed 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 (Unr, Brighton) for providing clinical strains of E. faecalis, GVH and BMRH for supplying valuable data for a study survey on burn wounds SPE pathogens. The authors also gratefully acknowledge the financial support from the Medical Research Council (MRC) through EPSRC (Grant numbers MR/J002536/1, MR/K006480/1) and the European Commission’s 7th Framework program (EC FP7 project no. 245500 Bacteriosense, the Healing Foundation for support via the Children’s Burns Research Center, the James Tait Foundation for support of research nurses BMRH, and the Anne Charlton Trust for PhD student support.

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