BACTERIAL PROTEASES: A MARKER FOR A ‘STATE OF PATHOGENESIS’ IN CHRONIC WOUNDS

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Introduction
Chronic wounds are open to the environment and are susceptible to contamination by bacteria, potentially infecting them. Some of the consequences of a chronic wound infection are tissue breakdown, pain, and adverse implications on the healing ability of the wound. Bacteria can cause disease, i.e. infection, which can be life-threatening.4 Identifying infection in chronic wounds is challenging because current clinical practice employs using clinical signs and symptoms (‘NERDS’) which are not necessarily distinct from other conditions, such as chronic inflammation. Bacteria and their proteases can stimulate a pro-inflammatory host response5 and, eventually, clinical signs and symptoms of infection1,9. Bacterial pathogenesis is undesirable since, at this stage, the wound is in a part of the wound infection continuum that typically requires intervention (figure 9).

Results
Results from Study 1 are summarised in figures 2, 3 & 4. The mean levels of pro-inflammatory cytokines IL-1β and TNF-α (figure 2) are significantly higher in wounds that are BPA positive versus wounds that are BPA negative. The host response often includes elevated inflammatory markers, e.g. cytokines including tumour necrosis factor alpha (TNF-α) and interleukin-1 beta (IL-1β). Unfortunately, clinical signs may not be apparent if the inflammatory response is impaired or defective (e.g. when other co-morbidities are present, such as diabetes or immunosuppressive conditions), whereby increasing the risk of infection.2,7 Bacteria are in a pathogenic state when they are either in the process of, or they are capable of, causing disease, i.e. infection. One indication of pathogenicity is the production of enzymatic virulence factors or bacterial proteases.6 The detection of bacterial protease activity (BPA) in a chronic wound would be indicative of the presence of bacterial pathogenesis which is a precursor to clinical infection.

Discussion
Increased production of pro-inflammatory cytokines (e.g. IL-1β & TNF-α) is known to be one of the host responses to infection by pathogenic bacteria.7 The results in figures 2 & 3 show that increased levels of IL-1β & TNF-α are detected when wounds test positive for BPA. However the data shown in figure 5 reveals that increased BPA can be detected even when the wound is asymptomatic for infection (i.e. only 2 ‘NERDS’ present). Therefore, the presence of bacterial proteases above a threshold level of activity can indicate to a clinician that the wound is progressing to the point when the host is mounting a biochemical response to the insult and the wound is developing towards infection (figure 9) that requires intervention even though overt signs are not obvious to the clinician.

Study 2: Bacterial protease activity in wounds exhibiting differing numbers of clinical signs of infection

Methods
Study 1: Duplicate swabs were taken from 186 chronic wounds (including LU, DFU, PU & other non-healing wounds) and compared to ensure homogeneity. One swab was extracted for testing in a laboratory protease assay using casein as substrate, including an inhibitor of human neutrophil elastase (‘Casein’ assay) and for analysis for bacterial protease activity (BPA).6 The second swab was tested on a prototype rapid lateral flow point of care bacterial protease test. The BPA is expressed as line intensity on the lateral flow test line evaluated using a visual analogue scale (VAS). Appearance of any test line (i.e. ≥0.25 VAS) on the bacterial protease test is indicative of presence of BPA.

Study 2: Wound fluid swabs were taken from 366 chronic wounds (including LU, DFU, PU & other non-healing wounds) were extracted for testing in the ‘Casein’ assay and assessment by the clinician for the number of clinical signs of infection (‘NERDS’). Wounds were classified as BPA positive if an Casian activity threshold (125 mU/ml/swab) was exceeded. An additional swab was taken for culture and quantitative microbiology.

Conclusions
Presence of pathogenic bacteria in a wound can cause a ‘state of pathogenesis’ that leads to local infection and impair healing. Clinical examination can wrongly diagnose infections in chronic wounds. Some chronic wounds fail to exhibit the classic signs of infection and inflammation in wounds can be misinterpreted as infection.10 Culture techniques have limited reliability on their own, frequently leading to the over diagnosis of infection. Therefore, testing wound fluid for BPA using a rapid point of care test may be a useful method for detecting the presence of pathogenic bacteria, at a clinically significant stage in the infection continuum, even before the signs of infection are apparent. Integrating a point of care test for BPA as part of routine wound assessment could be a valuable tool in treatment pathways to inform clinicians that the wound is in a ‘state of pathogenesis’ which could lead to overt infection. It is possible for a contributor to wound chronicity and have a negative effect on morbidity and mortality of the patient.

References
2. Carrington S, Smith KL. The role of endogenous and exogenous enzymes in chronic wounds: a focus on the implications of aberrant levels of both host and bacterial proteases in wound healing. Wound Repair and Regeneration 2012; 20: 125-36.
3. McCarty SM, Cochrane CA, Clegg PD, Percival SL. The role of endogenous and exogenous enzymes in chronic wounds: a focus on the implications of aberrant levels of both host and bacterial proteases in wound healing. Wound Repair and Regeneration 2012; 20: 125-36.
4. Unfortunately, clinical signs may not be apparent if the inflammatory response is impaired or defective (e.g. when other co-morbidities are present, such as diabetes or immunosuppressive conditions), thereby increasing the risk of infection. Bacteria are in a pathogenic state when they are either in the process of, or they are capable of, causing disease, i.e. infection. One indication of pathogenicity is the production of enzymatic virulence factors or bacterial proteases. The detection of bacterial protease activity (BPA) in a chronic wound would be indicative of the presence of bacterial pathogenesis which is a precursor to clinical signs and symptoms of infection.
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The prototype rapid lateral flow point of care bacterial protease test described in this poster is not FDA cleared for use in the US or any other part of the world.