Chronic wounds fail to progress through a normal and timely order of repair where infections along with other comorbidities hinder their normal healing process. The microbiology of chronic wounds is complex; it is believed that only when bacterial colonisation combined with other factors, such as decreased vascular supply, high bacterial virulence, and decrease in host immune factors can lead to wound infection.

Although wound infection is defined as the presence of >10⁵ colony-forming units (CFUs) per gram of tissue as demonstrated by biopsy in the international guidelines for pressure ulcers; latest studies have shown that bacteria are cultivated in less than 5% of chronic wounds, using traditional laboratory techniques.

The need for improved wound diagnosis at a localised level to predict specific microbial species in chronic wounds is recognised and outlined in the consensus document by the World Union of Wound Healing Societies. This discussion will examine potential novel biomarkers that could be the basis to develop a diagnostic test to detect microbial species in wounds by using wound fluid reverse transcription-polymerase chain reaction method (RT-PCR).

Research has confirmed that the gold standard for diagnosing wound infection using traditional laboratory techniques that depend on wound swabs and biopsies, are designed to detect only planktonic bacteria capable of being cultivated and not the bacteria present in poly-microbial communities and biofilms that are of high clinical relevance.

Recent research on using molecular diagnostics to identify bacterial DNA and RNA sequencing has shown its accuracy in detecting wound bacterial cells and determining their sensitivity to antimicrobial agents, compared with more established methods. Molecular analysis focuses on gene expression since evidence suggests that the host-pathogen interaction in wound infection can be assessed at the messenger-RNA (mRNA) level.

RT-PCR is a highly sensitive method for determining gene expression and wound fluids contain both bacterial and host cells, therefore it would be feasible that use of this test in combination with wound fluid could provide information to differentiate between infection and colonisation in vivo. This was examined in a study by Asada et al (2012) using an animal model. The authors hypothesised that using the RT-PCR method on the centrifugal precipitation of wound fluid could identify biomarkers to detect bacterial colonisation and infection in wounds. In this study, Asada et al (2012) created full-thickness wounds in rat models of the three groups of rates: control group, colonised group, and infected group, which were inoculated with different concentrations of Pseudomonas aeruginosa (P. aeruginosa) dispersion. After analysing the m-RNA expression in bacteria and host cells in the three groups, expression of bacterial housekeeping genes was detected only in the samples for the colonised group and infected group only. Expression of host housekeeping genes was detected in all the three groups. Expression of P. aeruginosa virulence factor was only detected in the samples from the infected group. Expression of the regulatory T cell-specific marker was only detected in the samples from the colonised group. These results show that the RT-PCR method could test and identify the combination of biomarkers that can differentiate between these three groups by analysing gene expression in their wound fluids. Despite these promising results, this study was conducted under highly standardized conditions using an acute animal model of wound infection with one strain of bacteria. Therefore further investigations are still required to address the other factors that may influence wound infection in clinical situations such as different chronic wound types, infections from other bacterial species and comorbidities.

Conclusion

Using wound fluid RT-PCR could be a useful approach in the search for new biomarkers to diagnose wound infection and be a foundation to create a point of care diagnostic test for early detection of pathogenic bacterial colonisation. Such a revolutionary shift from using older diagnostic methods to adopting molecular techniques could bring about change in infection control strategies and woundcare guidelines, and direct healthcare providers in managing wound infection using the appropriate treatment.

References