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Review Article

Wound diagnostics: Deploying electroanalytical strategies for point of care sensors and smart dressings

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Chronic wounds are highly heterogeneous with the complications of tissue remodelling and issues such as infection generating a multitude of molecular and cellular species. It could be anticipated that were information regarding the dynamics of key wound biomarkers available to the clinician, more informed decisions could be implemented to encourage the reinstatement of normal healing processes. There are few diagnostic options available at the time of consultation and the aim of this review has been to assess the capability of electrochemical sensing strategies to provide detailed point of care information on the wound condition. Advances in functional materials and the greater accessibility of disposable printed systems are beginning to provide a solid foundation through which low cost devices could be realised and whose deployment could lead to more informed decision making and positive outcomes.

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Introduction

Most wounds will heal with minimal intervention but the presence of a comorbid condition such as diabetes can give rise to complications that slow the healing processes or result in tissue regeneration becoming stalled [1–3]. Such chronic wounds can last from months to years generating painful and debilitating symptoms that invariably compromise the patient's quality of life [4]. It has long been recognised that the provision of detailed information on the activity of cellular and molecular species within the wound could enable more informed decisions and facilitate more timely interventions to enhance the healing processes [3]. Such tests however are seldom available to the clinician and, where they are, it is invariably through a central laboratory [2–4]. The situation is further complicated when considering recent UK estimates that indicate some 74% of wounds are presently treated in the community [4•]. This places the onus of diagnosis on the vigilance of the visiting healthcare worker and the patient which can lead to excessive time delays in reporting concerns and receiving the treatment. It is little surprise that the availability of low cost diagnostic devices capable of capturing detailed concentration profiles of the key biomarkers associated with wound repair at the point of care have long been an aspiration of clinicians [3,5,6]. The increasing accessibility of disposable electrodes has provided a golden opportunity for the electroanalytical techniques to step into the breach providing rapid quantitative analysis [5]. There are however many challenges with selectivity and sensitivity being key concerns. The primary aim of the present communication has been to cast a spotlight on a selection of clinical targets, their function and the approaches presently being taken in development of point of care sensors.

Wound fluid biomarkers – potential targets

Given the initial trauma to the tissue and the subsequent tissue remodelling and inflammatory responses that unfold with the wound, there is a vast number of biomolecular species that have some part to play in the healing processes [3,5–7]. A snapshot of some of these players is provided in Table 1 [7]. Identifying markers that can provide key warnings towards the onset of complications, particularly infection, and subsequently developing procedurally simple, low cost sensors that can enable their robust quantification are the core challenges [5,6].

There are two approaches to the development of wound diagnostics – disposable standalone tests and wearable devices that can be provide remote telemetry of the wound environment [5,6]. The latter has tended to be described as a smart dressing or bandage and although it represents an idealised concept, its realisation is incredibly more complex than the development of single shot *in vitro* sampling devices. Sridhar and Takahata were among the first to produce a device capable of wirelessly monitoring wound pH with a pH sensitive hydrogel sensor [8] but there has been relatively few developments since. Wang and coworkers followed with an enzyme

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Table 1

Potential wound biomarkers (Adapted from BroadBent *et al.* [7]).

α-amylase	IgA
α-1-antitrypsin	IgG
α-1-acid glycoprotein	Insulin-like growth factor -1
α-1-globulin	Insulin-like growth factor binding protein-2
α-2-macroglobulin	Insulin-like growth factor binding protein-3
Alanine aminotransferase	Inter-α-inhibitor
Albumin	Interferon-inducible protein-10
Alkaline phosphatase	Interleukin-1, 1a, 1b, 6, 8, 10
Angiostatin	Interleukin-1 receptor antagonist protein
Antichymotrypsin	Keratin 6
Apolipoprotein A-1	H, L-Kininogens
Asparate	Lactate Dehydrogenase
aminotransferase	**Lysozyme**
β-1-globulin	Macrophage inflammatory protein 1a, 1b
β-globulin	**Matrix metalloproteinases -1, 2, 8, 9**
β-2-glycoprotein-1	**Myeloperoxidase**
Basic fibroblast growth factor	Monocyte chemoattractant protein 1
Calgranulin A and B	Neutrophil cathepsin-D
Cathepsin G	**Nitric oxide**
Ceruloplasmin	Nucleosome assembly protein 2
Collagen I and III	Orosomucoid 1
Complement C3 and C4	Oxygen
C-reactive protein	p55, p75
Creatine kinase	**pH**
Cytokeratin-1	Plasmin
Elafin	Plasminogen
Elastase	Plasminogen activator
Elastase (Neutrophil)	Plasminogen activator inhibitor
Endostatin	Platelet derived growth factor - AA, AB
Epithelial growth factor	Platelet factor-4
Epithelial neutrophil activating peptide - 78	**Pyocyanin**
Factor B	Tenascin-C
Fibrinogen-α, b gA chains	Tetranectin
Fibronectin	Tissue inhibitor of metal proteinases-1 and 2
Ferritin	Tumor necrosis factor-α
γ-globulin	Transferrin
γ-glutamyltranspeptidase	Transforming growth factor-β, B1
Glypican-1	**Uric Acid**
Granulocyte macrophage colony stimulating factor	Urokinase plasminogen activator
Growth regulated oncogene-α	Vascular endothelial growth factor
Haemopexin	Vitamin D binding protein
Hepatocyte growth factor	Vitronectin
Haptoglobin	
Heparin binding protein	**(Bold: Existing Electrochemical Targets)**

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sensor for the measurement of wound urate [9••] and Farooqui and Shamim extended the approach by integrating pressure and pH measurements [10••]. In most approaches, the rationale has been to rely on a screen printed disposable dressing with nondisposable electronics completing the "smart bandage". The complexity of design involved in integrating the sensing and reporting components can be considerable hurdles especially when considering that the sensor component must be disposable. It is little surprise therefore that the move from bench top investigations to wearable devices with wireless reporting capability has been very slow. As such, most of the developments within wound diagnostics, especially those targeting proteins, have tended to been focused on single assay systems. This is far from the robust multi-parametric molecular profiles required to meaningfully understand the wound dynamics but, could be invaluable in raising an alarm to the onset of infection. A notable exception however is the microfluidic systems being developed by Rusling and coworkers who have demonstrated the detection of multiple cancer biomarkers at screen printed carbon and gold arrays [11••] and it could be envisaged that such technologies could equally be applied to wound diagnostics.

Despite the availability of numerous biomolecular targets, electrochemical approaches to wound sensing have tended to focus on a very limited subsection: pH, urate, nitric oxide and immune response proteins [5,6]. The measurement of wound pH was one of the first parameters to catch the attention of the electroanalytical community given the extensive literature base already devoted to pH sensing. A multitude of designs have been evaluated in single shot, periodic and continuous sampling formats [5,6]. While voltammetric and potentiometric methodologies have been assessed [12], it is the latter that continues to generate most interest. The efficacy of using polyaniline as the pH sensitive element in a wearable dressing was first demonstrated by Wang and coworkers [13] and has continued to be adapted for use in a variety of disposable sensing formats [14,15]. The most recent approach has seen the polymer being integrated within nano-pillar formats which have exhibited excellent stability (drift: < 1 mV/12 h) and mechanical flexibility – both being critical design prerequisites for providing dressings capable of remote telemetry [15•]. Inkjet palladium/palladium oxide composites [16] and field effect transistor (FET) devices [17] have also emerged as potential routes to monitoring pH.

Detection of immune response proteins

The ability to extract a more complete picture of the wound dynamics in the clinic or within home health settings could dramatically improve care [3]. This is particularly true in the case of infection where early detection is a key factor in improving outcomes and where delays in responding could lead to a wound becoming irretrievably damaged with limb or life threatening consequences [2–4]. The expression of immune response proteins is one of the most sensitive indicators of early infection and therefore, monitoring sudden increases in their production or activity could provide an indication that the wound condition had changed from benign contamination to critical colonisation and infection [3]. C-Reactive Protein (CRP) [18–20], Matrix Metalloproteases (MMPs) [21–25], Human Neutrophil Elastase (HNE) [26,27] and Lysozyme [28–32] have all been shown to have diagnostic merit in the management of chronic wounds and there are numerous ELISA's that can provide quantitative data [7]. The latter are almost invariably complex multistep processes which are centralised and will inevitably incur delays in analysis and reporting.

Immunochromatographic (lateral flow) devices can be conducted at the point of care but, although offering a more rapid response, suffer in terms of a qualitative output. The translation of ELISA systems to an electrochemical format is possible but their complexity is problematic from the perspective of implementing it as a screening tool directly within clinical practice. As such, there is an increasing pursuit of label free strategies in a bid to retain the simplicity of the lateral flow systems whilst offering high sensitivity. These typically employ screen printed systems, FETs or quartz microbalances upon which the antibody or aptamer is immobilised. These are proven technologies but recent innovations have focused on the integration of graphene, graphene oxide and gold nanoparticles to facilitate immobilisation of the recognition element (antibody, aptamer or peptide sequence). Such modifications can also enhance the electron transfer of redox probes (typically ferrocyanide) at otherwise unresponsive printed substrates providing greater clarity (magnitude and resolution) of the voltammetric signals at potentials where there may be less interference from the matrix constituents [33] . A summary of the different label free approaches to the detection of the immune response proteins identified previously is presented in Figure 1 and each is discussed in turn.

CRP is one of the most commonly requested clinical markers where a normal level of 5 mg/L can rise significantly upon infection to more than 100 mg/L [34]. Thus a sensor able to determine low concentrations could be useful in the diagnosis of acute phase infection [3]. The rapid accumulation of this protein however also requires a large dynamic range such that the response to the subsequent treatment and recommencement of healing can also be gauged. Impedimetric immunoassays have a long track record in the detection of a wide range of proteins and could be applied to many of those listed in Table 1. Yagati et al. demonstrated the applicability of the approach to CRP with a patterned ITO electrode array and exploited the enhanced electron transfer properties of reduced graphene oxide and gold nanoparticles immobilised along

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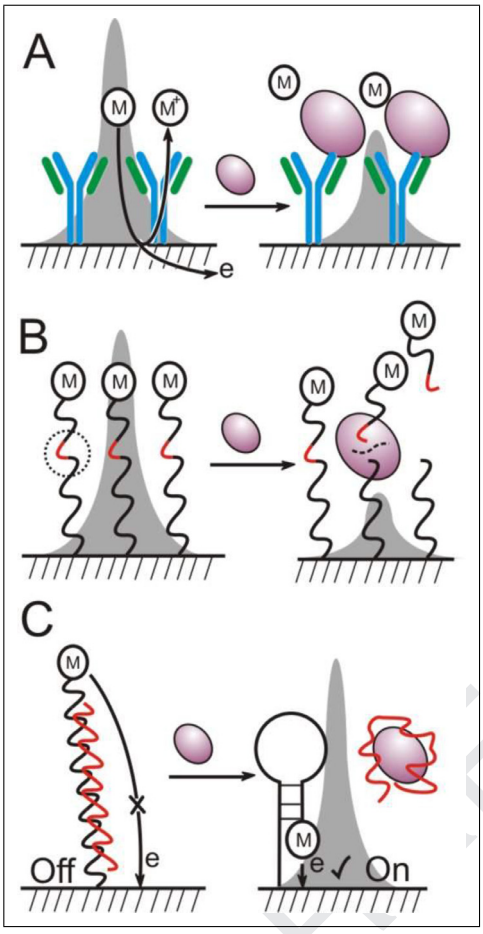
Figure 1



Principal electroanalytical detection pathways for immune response proteins. A) Generic label free immunoassay. B) Redox probe labelled peptide with sequence-specific enzyme promoted hydrolysis. C) Redox labelled aptamer recognition.

with the antibody [18]. An alternative approach however has been promoted by Goda and coworkers through the use of a Poly(3,4-ethylenedioxythiophene) film functionalised with a zwitterionic phosphorylcholine moiety [19]. The latter serves as a biomimetic ligand for CRP and, although somewhat radical in its nonantibody design, detection of the binding event is more conventional with impedimetric or voltammetric methodologies employing ferrocyanide as redox probe.

MMPs are responsible for remodelling the extracellular matrix within the wound through degrading protein matter but, when there is disease or extensive inflammation, the normal checks and balances can fail, resulting in the over expression of MMPs [2,3]. Thus, an abnormally high MMP activity leads to the dismantling is often used as a critical diagnostic of poor wound healing and there are a number of commercial immunoassays based on lateral flow technologies and targeted specifically at chronic wounds (i.e. Systagenix Woundchek™). A number of electrochemical assays have arisen and while some exploit the simple impedimetric format described for CRP [21], others have sought to take advantage of the enzymes native protease action [22–25]. The immobilisation of specific peptide sequences onto electrode substrates serves as the sensing target which, when exposed to MMP, are cleaved at a specific point. The hydrolysis process will be dependent on MMP activity and easily monitored through changes in impedance [22,23]. The addition of a methylene blue redox label at the peptide terminal has also been used with cyclic voltammetry employed to monitor the MMP induced loss of the probe [24]. The cleavage approach was also adopted by Kou but supplemented with the incorporation of a highly intricate enzyme linked DNA nano-ladder and electrocatalytic substrate [25••]. The approach is notable in its sophistication but such complexity is liable to be economically and procedurally prohibitive.

Neutrophil granulocytes will be among the first cell type to appear within the wound bed and are the host's primary form of decontaminating the wound. The neutrophils release a cocktail of enzymes of which HNE will be one of the most abundant in chronic wounds [35]. Bopp and Goynes were among the first to develop HNE assays and exploited the protein's sequence-specific hydrolysis of peptides to release a chromophore [26]. Such approaches have continued to be optimised [27] and it could have been anticipated that the translation of the approach to an electrochemical assay would be inevitable. The basic approach is little different from the detection pathways highlighted for MMP but, surprisingly, there are no HNE electrochemical assays.

Lysozyme is responsible for responsible for breaking down the polysaccharide walls of certain bacteria and is one of the body's central defences against bacterial infection [28–31]. Aptamer recognition has been the foundation of lysozyme detection though the design of the latter has been regularly updated with a number of methodological advances. Impedimetric analysis [28], chronopotentiometric [29] and voltammetric [30–32] detection of conformational changes of the nucleic acid have all been investigated. The major methodological development in recent years however has been the labelling of the nucleic acid with a redox probe (methylene blue) such that upon interaction with the lysozyme, the sensor is switched to an "on" state [31••,32•]. In the absence of the biomarker – the redox probe is spatially removed from the underlying electrode but the lysozyme induced conformational change results in the probe being sufficiently close to allow electron transfer and, in contrast to many earlier protocols, the signal increases with increasing lysozyme.

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Summary

The increasing focus on the design of label free detection strategies for protein species and the increasing availability of low cost disposable electrodes are significant factors in the realisation of devices that could be used directly within the clinic. The clinician's dream of rapid multiparametric analysis is still some distance away but as electrochemical assays become more robust, fulfilling such a goal (albeit in a more modest form) will undoubtedly be the next challenge.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest.

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