Sensors and Imaging for Wound Healing: A Review

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Abstract (150-250 words)

Wound healing involves a complex series of biochemical events and has
traditionally been managed with 'low tech' dressings and bandages. The concept
that diagnostic and theranostic sensors can complement wound management is
rapidly growing in popularity as there is tremendous potential to apply this
technology to both acute and chronic wounds. Benefits in sensing the wound
environment include reduction of hospitalization time, prevention of
amputations and better understanding of the processes which impair healing.
This review discusses the state-of-the-art in detection of markers associated
with wound healing and infection, utilizing devices imbedded within dressings
or as point-of-care techniques to allow for continual or rapid wound assessment
and monitoring. Approaches include using biological or chemical sensors of
wound exudates and volatiles to directly or indirectly detect bacteria, monitor
pH, temperature, oxygen and enzymes. Spectroscopic and imaging techniques
are also reviewed as advanced wound monitoring techniques. The review
concludes with a discussion of the limitations of and future directions for this
field.
1. **Introduction**

In the last century, there have only been a handful of technical advances that have contributed to changes in the discipline of wound management. One of the most important was in the 1960s when it was found that keeping a wound moist accelerates the healing processes. This was reported in the pivotal study by Winter in 1962 (Winter 1962) and later became accepted practice and indeed a key design parameter in the development of dressings (Wu et al. 1995). The other crucial developments have been the management of infections in wounds through the use of anti-microbial agents, most notably, silver and iodine (Mertz et al. 1999; Wright et al. 2002), the use of compression pressure therapy (for chronic venous leg ulcers) (Wong et al. 2012), skin grafts (Rizzi et al. 2010) and hyperbaric oxygen therapy (breathing 100% oxygen at elevated pressure) (Malda et al. 2007). Each of these breakthroughs has resulted in new commercial
products. For instance, there are now numerous different hydration-controlling dressings available (Queen et al. 1987; Queen et al. 2004); while for infection control dressings impregnated with silver have become ubiquitous in wound management (Wright et al. 2002) (Figure 1). Despite these advancements and the wide range of dressings available, wound management is still extremely challenging due to its subjectivity, the complexity of the wound healing process itself, and patient variability.

There is now growing evidence to suggest that we are currently on the verge of the next significant advance in wound care where sensors will be used as diagnostic tools in wound healing to revolutionize wound care practice. The main types of wounds which will benefit most from sensor technology are chronic ulcers, and to a lesser extent infected acute wounds and large full-thickness burns. Chronic ulcers can be especially difficult to treat, highly susceptible to infection and can cause long-term suffering for the patient. Sensor innovation in the management of these wounds has the potential to impact clinical practice, patient outcomes and economic policy.

Chronic ulcers are a substantial cause of morbidity and societal burden worldwide. Foot ulcers, for instance, occur in approximately 25% of diabetics and are the leading cause of non-traumatic amputation in developed countries (Turns 2011). In the United States, chronic wounds as a whole are estimated to affect approximately 1–2% of the population during their lifetime (Gottrup 2004), translating to an estimated morbidity of 6.5 million sufferers at a cost of US$25 billion per annum (Crovetti et al. 2004; Singer and Clark 1999). Equally concerning is the rate at which chronic wound incidence is increasing due to lifestyle changes and the aging population. Together these phenomena pose a substantial threat to the future of health provision and the management of national economies (Sen et al. 2009). Wound management technologies and strategies are underdeveloped and need to evolve to meet this present and increasing challenge. Accordingly, reflecting on current practice may identify avenues for innovation and improvement.

Currently, when a patient presents with a wound there are a series of standard steps that will be followed by the clinician. Initially, their past medical history, including cardiovascular profile and peripheral vascular disease, and the
wound history would be established together with a physical examination of the patient and of their wound (Turns 2011). This may be followed by an array of tests including physical examination and imaging of the wound. In a best-case scenario once the wound aetiology and the current status of the wound have been established, a management plan will be put in place. This can involve swabbing for detection of infection, preparation of the wound (cleaning and possibly debridement), dressing of the wound and coverage with bandages.

The time taken from initial presentation of a wound to the commencement of a wound management plan may be lengthy and require multiple appointments with a clinician, as any laboratory tests ordered can take hours or days to complete. Once treatment has commenced the patient would then be re-evaluated at subsequent appointments which can be days to months apart. These cumulative delays and follow-up appointments stall the administration of appropriate treatment and lead to increased cost. This is critical in terms of chronic wound management, where it has been shown that the longer the delay in treatment, the more difficult a wound is to heal (Margolis et al. 2000; Moffatt et al. 2009). As such, the use of rapid, specific and quantitative assessments, that can be completed during a standard medical consultation, would be better suited to wound management.

Point-of-care (POC) technologies are designed to provide rapid medical assessments at, or near, the site of patient care. These technologies are well suited to wound management due to their designed ease-of-use, convenience and rapid turnaround. In terms of wounds, POC assessment has the potential to ensure that effective management, based on relevant biochemistry, is administered without delay, rather than after the fact. This type of assessment is further suited to wound management due to the abundance of assayable biochemistry found within the wound environment such as proteolysis and reduction-oxidation events. Tests could be modelled from the modern pregnancy test whereby a simple readout is diagnostic of a particular wound parameter. Indeed, this has been the approach of Systagenix in the development of a POC device for evaluating elevated protease activity through their Woundcheck™ Protease Status diagnostic (Serena et al. 2011) (Figure 1). While POC technology, such as that developed by Systagenix, is well-suited to wound
management, technological innovations are paving the way for the development of next-generation wound assessment tools.

An area of growing research which could revolutionize wound care is in ‘smart dressings’. Similar to the emerging field of “wearable” or “epidermal” electronics (Kim et al. 2011), smart dressings use biochemical cues to generate readable output which has diagnostic or theranostic value. The difference between these two terms is that a diagnostic will give an accurate, non-subjective decision-making output (e.g. the wound is or is not infected), whereas a theranostic may help guide treatment (e.g. the wound has a certain level of matrix metalloproteinases) (Harding 2007). Diagnostics would be aimed at the deskilled care-giver, whereas theranostics would be useful for experienced wound specialists and researchers. ‘Smart dressings’ have the potential to expedite the diagnosis of wounds through the use of biosensors either incorporated into or near wound dressings, similar to POC dip-stick-type tests, to generate a result in minutes. When applied as continuous or semi-continuous monitoring devices, smart dressing readouts may also be used to resolve predictive trends. The ability to not just monitor wound healing but also to predict it will help ensure potentially problematic wounds receive the appropriate attention at the earliest possible stage. This information may be used to help guide wound practitioners to adopt a more or less aggressive management strategy, at the right time, to achieve healing.

As ‘smart dressings’ and POC diagnostics/theranostics are on the verge of becoming a commercial reality, it is an opportune time to review the literature in order to examine the wide range of approaches being used by multiple disciplines to apply sensor and imaging methods to the wound environment. While there has been extensive research into sensors for biomedical applications, this review will be restricted to technologies pertaining to wound healing.

2. Potential markers

The complexity of the wound healing process means there ought to be an abundance of possible diagnostic and theranostic targets. A list of markers under
investigation for use in wound healing was recently published by Harding and an expert working group in “Diagnostics and wounds – a consensus document” (Harding 2007) and is reproduced in Table 1 below.

Table 1. List of potential markers for wound healing (Harding 2007).

- Bacterial load/specific microbial species/biofilms
- Cytokine release in response to specific microbial antigens
- DNA – e.g. gene polymorphisms to indicate susceptibility to disease, poor healing or infection
- Enzymes and their substrates – e.g. matrix metalloproteinases and extracellular matrix
- Exposed bone
- Growth factors and hormones – e.g. platelet-derived growth factor (PDGF), sex steroids (androgens/oestrogens), thyroid hormones
- Immunohistochemical markers – e.g. integrins, chemokine receptors and transforming growth factor beta II receptors to monitor healing status
- Inflammatory mediators – e.g. cytokines and interleukins to monitor healing status and guide use of anti-inflammatory treatments
- Nitric oxide
- Nutritional factors – e.g. zinc, glutamine, vitamins
- pH of wound fluid
- Reactive oxygen species
- Temperature
- Transepidermal water loss from periwound skin

What is noticeable about this table is that it relatively general and highlights the importance of continued research into better understanding the specific markers involved in wound healing. This is not to say that the search for markers of chronic wound healing has been stagnant. Many of the biochemical components found in Table 1 are underpinned by numerous clinical investigations involving the analysis of wound tissues and exudates (Broadbent et al. 2010). These investigations detail the analysis of over 150 proteins and metabolites and, despite limitations in sample number or exclusion criteria (Liu et al. 2011), are the foundation of our current understanding of chronic wound biochemistry. Noteworthy examples include the identification of over-abundant proteases and their therapeutic and prognostic potential (Cao et al. 2011; Liu et al. 2009), and identification of growth factor dysregulation (Cooper et al. 1994) and the potential to restore healing through their inactivation (Streit et al. 2006).

Regardless of these successes, wound investigations are yet to result in the
identification of specific and quantitative targets for use in clinical practice (note that the Systagenix elevated protease activity diagnostic is not currently used in clinical practice). Concomitantly, chronic wound biochemistry is generally still regarded as controversial (Liu et al. 2011).

Recent literature has provided new insights into mechanisms taking place in the chronic wound environment. Work by Fernandez et al. (2012) has demonstrated a correlation between uric acid concentration and wound severity in clinically uninfected wounds. The authors further showed the concurrent depletion of uric acid precursors and demonstrated the ex vivo activity of the responsible enzyme, xanthine oxidoreductase, in wound fluid. (Hoffmann et al. 2011) investigated the association of the protease inhibitor, α1-antichymotrypsin, with wound healing and was able to show a functional relationship between its inactivation and wound healing. Both of these investigations outline mechanisms for inflammatory mediation and offer potential targets for the development of innovative sensing devices. In addition to these autogenic molecular targets, complications such as infection have been sufficiently examined in other medical contexts to be the focus of sensor development for wound management.

3. Sensors for detection of infection

One of the single most common complications preventing wounds from healing is infection. Staphylococci and Streptococci are the two prevalent opportunistic pathogenic organisms found in community-acquired superficial wounds and are also common to many chronic wounds (Davies et al. 2001) known to harbour diverse microbial populations (Dowd et al. 2008). There are many obvious signs of advanced infection including redness, heat, swelling, purulent exudate, smell, pain, systemic illness, “foamy” granulation tissue, contact bleeding, tissue breakdown and epithelial bridging (Grey and Harding 2006) but the challenge is to detect infection before it reaches this stage.

The current method for characterization of infection is usually taking a swab from the wound site. The swab is then analysed in a microbiology laboratory for bacterial growth (e.g. semi-quantitative culture) and graded on a
scale ranging from “scanty” through to “heavy”. There is frequent prejudice towards motile and fast growing organisms while fastidious organisms, such as anaerobes, may be under-represented (Healy and Freedman 2006). Swabs can often only detect superficial pathogenic organisms and fail to indicate what is happening deeper in the wound. Other analyses might include gram stain and quantitative culture. Most wound swabs are prone to false positives as most will yield bacterial growth which is not always due to infection. Treatments may include antiseptic topical treatment with silver compounds or iodine or systemic treatment with antibiotics. While designed to inhibit growth of micro-organisms, the compound-loaded dressings can also lead to impairment of functions important to wound healing. For instance, the silver-loaded dressing Acticoat has been shown to cause impairment of skin cell proliferation and function in addition to its desired microbicidal activity (Poon and Burd 2004).

There is a clear need for methods to enable early detection of infection to guide the use of antibiotic treatments. Moreover, indication of when treatment is not required would be valuable in reducing costs and overuse of antibiotics and antibacterial substances (e.g. silver) leading to resistant strains and delayed healing, respectively (Burd et al. 2007). The large number of markers for infection has led to many diverse approaches by researchers to use sensors either incorporated into dressings or as POC tools.

3.1 Sensors targeting bacterial biochemistry

Bacteria secrete a variety of tell-tale biochemical by-products. For instance, pyocyanin - the blue-green pigment secreted by most Pseudomonas (Ps.) aeruginosa strains, has been used by Sharp et al. (2010) to indirectly detect bacteria. Using a carbon fibre tow consisting of several thousand carbon filaments with diameters of 10 μm thermally sandwiched into a lamination pouch, they were able to observe the oxidation and reduction events of pyocyanin using square wave voltammetry. This allowed them to reproducibly quantify its presence in buffered solutions over a concentration range of 1 – 100 μM. Furthermore, they were able to monitor pyocyanin in broth culture of Ps. aeruginosa using the carbon fibre electrodes and observed good correlation with a standard chloroform-acid photometric method for quantification. Advantages
of this method include a small voltage scan range which limits possible interference from endogenous compounds, small/cheap electrode materials which can be incorporated into existing dressings or bandages and the possibility for early detection of colonising *Ps. aeruginosa*. The obvious disadvantages are that it requires a power source and an electrochemical detector.

Urate or uric acid is another metabolite found within wounds which may be used as a diagnostic marker for colonization of *S. aureus* and *Ps. aeruginosa*. It is believed these bacteria rapidly metabolize uric acid via uricase synthesis, therefore a rapid decrease in uric acid concentration within wound fluid may be a generic indicator for bacterial colonization. In a similar approach to the pyocyanin sensor above, Sharp et al. (2008) used a carbon fibre sensor laminate and square wave voltammetry to detect the oxidation of urate at +0.23 V as a sharp peak free from interference from other metabolites. Successful measurements in blood and blister fluid were also reported although problems were encountered with loss of signal over time due to electrode fouling. This was partially solved by using a cellulose acetate barrier around the electrodes to filter out large proteins and fats thought to be responsible for the fouling. While generally applicable to acute wounds, this diagnostic test may suffer in its application to chronic wounds. Work by Fernandez et al. (2012) recently showed that chronic venous leg ulcers, with no clinical sign of infection, actually had elevated uric acid concentration. Furthermore, its concentration correlated positively with wound severity. The increase in uric acid in severe chronic wounds and the decrease in infected wounds clearly indicates the biochemical complexity of this approach.

The toxins produced by bacteria found in wounds may be used to trigger a sensor output indicating early infection. Inspired by the mechanisms in which toxins phospholipase A2 and α-hemolysin from *S. aureus* and *P. aeruginosa* permeabilize or hydrolyze cell membranes, Zhou *et al.* reported synthetic Trojan-like phospholipid vesicles, which are disguised as eukaryotic cells but release a fluorescent dye cargo when acted on by these toxins (*Figure 2*) (Zhou et al. 2010; Zhou et al. 2011). They achieved this by synthesizing phospholipid-based giant (ca. 50 μm) unilamellar vesicles containing the dye
carboxyfluorescein which were stabilized by UV crosslinking of a photopolymerizable fatty acid and immobilized onto surface-modified polypropylene fabric. Proof-of-principle experiments incubating the fabric-bound vesicles with *P. aeruginosa* and *S. aureus* demonstrated emission of green fluorescent light on irradiation with low intensity UV light while the control incubated with *E. coli*, which does not produce the lysing toxins, did not produce significant fluorescence. This same approach was used to release the antimicrobial sodium azide from the vesicles (Zhou et al. 2010), further increasing their use as a responsive dressing. This work according to the authors is continuing and is now investigating sensitivity to a variety of strains of *P. aeruginosa* and *S. aureus* (Figure 2) (Zhou et al. 2011).

Neutrophils are a cell type other than the bacteria themselves that are a marker for infection. In normal wound healing, neutrophilic granulocytes are rapidly recruited to the site of injury where they release enzymes to purge the wound of necrotic tissue. Once the wound has been cleansed, the neutrophil activity should cease. Infection also stimulates neutrophils and while they may help fight off infection there is evidence that they prevent wound closure through the degradation of important growth factors and ECM proteins (Yager et al. 1997). Two of the enzymes excreted by neutrophils, namely, human neutrophil elastase (HNE) and cathepsin G (CatG), have recently been reported as early stage warning markers for wound dressing-based diagnostics by Hasmann et al. (2011). They used an approach similar to an earlier report by Edwards et al. (2005) which exploited the enzymatic nature of HNE and CatG towards peptide substrates to produce a sensor made from a chromophore linked by a short peptide sequence susceptible to cleavage by each of the enzymes. The chromophore/peptide combinations used were N-methoxysuccinyl-Ala-Ala-Pro-Val-*p*-nitroanilide (MeOSuc-AAPV-pNA) and N-Succinyl-Ala-Ala-Pro-*p*-nitroanilide (Suc-AAPF-pNA) which are hydrolyzed by HNE and CatG, respectively. These were immobilized onto several typical wound dressing materials: silica gel, collagen, collagen/hyaluronic acid (with and without thiol groups), polyamide and polyethylene terephthalate. These dressings were incubated with fluid from either infected or non-infected wounds and monitored by UV absorbance for detection of the cleaved *p*-nitroanilide. In
all cases the fluid from infected wounds led to greater absorbance measurements which can be correlated back to increased HNE or CatG (silica gel only) concentrations for the respective peptide sequences. It was argued that while this approach requires long incubation times (12+ hours) the fact that the substrates were incorporated into the dressings constantly in contact with the wound, these times were not prohibitive. Much shorter incubation times of 2-5 minutes have been reported by Edwards et al. (2008) using cellulose-AP-suc-Ala-Ala-Pro-Ala-pNA substrate. A dip-stick approach with the same types of substrates would be much more acceptable if the incubation time were within the time of a patient consultation.

The group of Rimmer has recently reported an elegant method for binding either gram positive or gram negative bacteria to a polymer which changes conformation upon binding and has potential as a bacterial sensor. Highly branched poly(N-isopropylacrylamide) (HB-PNIPAM) was modified with the peptidic antibiotics polymyxin or vancomycin which bind to the cell membranes of gram negative (e.g. *Ps. aeruginosa*) or gram positive (e.g. *S. aureus*) bacteria, respectively (Sarker et al. 2011; Shepherd et al. 2010). When incubated with their bacterial targets, the HB-PNIPAM-polymyxin and HB-PNIPAM-vancomycin conjugates changed from soluble open coil structures to agglomerated globule structures which were used to deplete bacteria from an infected 3D tissue engineered skin model (Shepherd et al. 2011). This same approach may be used to selectively detect bacteria by incorporation of a fluorescent probe.

### 3.2 Porous silicon

Porous silicon (pSi) has become a popular substrate for optical biosensor manufacture and may have potential application in wound sensors. A number of specific characteristics underscore the popularity of pSi including: cost-effectiveness; ease of use in fabrication via anodization of semiconductor grade silicon; flexibility in fabricating different architectures including multilayers of alternating low and high porosity (through adjustment of etching parameters) (Jane A. et al. 2009); a well understood chemistry for biofunctionalization
(Stewart and Buriak 2000); and, tuneable optical properties. The nanoscale pores and crystalline features of the pSi lead to quantum confinement effects causing visible spectrum luminescence (Canham 1990). Furthermore, the large surface area of pSi nanostructures is well suited to the detection of changes in refractive index, leading to the prospect of gas sensing or biosensing (Jane A. et al. 2009; Letant and Sailor 2000; Worsfold et al. 2006). In fact, the optical reflectance properties of the thin pSi film result in the appearance of Fabry-Pérot interference fringes. Changes in the effective refractive index of a thin pSi film cause a shift in the interference pattern and affect the position of the photonic resonance peak in multilayered pSi resonators. Hence, capture of biomolecules within the film alters the optical properties of the film, in some cases even visible to the unaided eye, and afford the transduction of the binding event (Amato and Rosenbauer 1997; Bisi et al. 2000; Chazalviel and Ozanam 1997; Fauchet 1998; Ouyang et al. 2005; Sailor and Link 2005; Vincent 1994).

To demonstrate pSi as a substrate for bacteria common to wounds the groups of Miller and Fauchet coated a photoluminescent pSi microcavity with a peptidomimetic receptor for diphosphoryl lipid A, a component of lipopolysaccharide found in the outer cellular membrane of gram negative bacteria. When exposed to *E. coli*, *Salmonella* and *Ps. Aeruginosa* (all gram negative), a red shift in the photoluminescence spectra was readily observed spectroscopically, whereas the photoluminescence was not affected when exposed to gram positive bacteria *B. subtilis* and *L. acidophilus* (Figure 3a) (Chan et al. 2001; Fauchet 1998; Ouyang et al. 2005).

Biosensors adapted to cell and tissue culture situations have substantial potential to not only detect the presence of certain cells but also changes in cell behavior, which is highly relevant to wound healing (Chan et al. 2001; Li et al. 2011; Massad-Ivanir et al. 2011; Massad-Ivanir et al. 2010). Schwartz et al. (2006) have monitored the health of prokaryotic and eukaryotic cell cultures by means of light scattering from the surface of a pSi resonator where the introduction of a scattering centre, such as a bacterium or a mammalian cell resulted in oblique angle detection of a pSi resonance. This concept was used to monitor physiological changes occurring in primary rat hepatocytes that affected their viability (Schwartz et al. 2006) and to monitor the proliferation of
*Pseudomonas* bacteria and their lysis upon viral infection in real time (Alvarez S. D. et al. 2007).

Recent studies have also demonstrated the detection of proteases using pSi resonators. Matrix metalloproteinases (MMP) are zinc-dependent endopeptidases with increased activity in chronic wound fluid (Rayment et al. 2008). Gao *et al.* sensed MMP-2 at concentrations as low as 0.1 ng/mL by spin-coating a protective gelatin film on top of a pSi resonator (Gao *et al.* 2008). Upon contact with MMP-2, the film was digested and the resulting peptides were able to enter the pores and induced color changes visible to the unaided eye due to red shifts in the position of the photonic resonance (Figure 3b). Likewise, Orosco *et al.* (2006) obtained red shifts in resonance peaks upon digestion of spin-coated corn protein (zein) films over a pSi resonator in the presence of pepsin. In contrast, Kilian *et al.* (2007) exploited a blue shift upon release of peptide fragments from angiotensin immobilized into a pSi resonator in the presence of the endoprotease subtilisin. Similarly, Martin *et al.* (2010) developed a pSi microcavity to detect MMP-8. Instead of exploiting the enzymatic activity of MMP, the researchers attached anti-MMP antibodies on the walls of pSi and were able to detect the presence of MMP-8 at the level of 100 ng/mL through immune-capture into the pores.

In summary, pSi based structures capable of detecting bacteria and enzymes via shifts in their photonic properties lend themselves to the development of stand-alone optical sensors which could be incorporated into dressings, such as hydrogels (Jane A. *et al.* 2009) to provide real-time data via colorimetric readouts.

### 3.3 Odor Sensors

Artificial olfaction systems, commonly referred to as electronic noses, have long been used to detect volatile chemicals produced by bacteria (Allardyce *et al.* 2006; Pavlou *et al.* 2002; Wiggins *et al.* 1985). Many of the by-products of bacterial metabolism are airborne (spores, volatile organic compounds and mycotoxins) and have characteristic odors which can give clues to the identity of the bacteria, for instance specific anaerobic bacteria give rise to unpleasant
malodor (Bowler et al. 1999) The advantage odor sensors have over dressing-bound detection systems is that they can be used in situations where the dressing is under layers of compression bandages which, to be effective, need to be kept in place for long periods without being disturbed.

Tandem gas chromatography–mass spectrometry (GC-MS) is the traditional method for analysis of volatile species but the instrumentation is bulky and very costly. The common alternative is to use solid-state gas sensors which rely on a change in conductivity, capacitance, work function, mass, optical and reaction energy which can vary depending on the gas-solid interaction (Korotcenkov 2007). The common types of gas sensors used as odor sensors in medical applications have recently been reviewed by Persaud (2005) and vary in specificity, portability and cost. While gas sensors have attracted intense interest in disease and biosecurity applications, only a few reports have addressed their use in wound healing. To be successful, it has been suggested that a database of wound volatiles is required, together with technology miniaturisation and cost reduction (Thomas et al. 2010).

The volatile organic compound profile emitted by chronic wound lesions can be extremely complex. Polydimethylsiloxane skin-sampling patches fed into GC-MS revealed over 300 resolved peaks and many unresolved peaks from venous ulcer samples (Thomas et al. 2010). While GC-MS analysis generates a wealth of data, the cost and bulk of the instrumentation makes it prohibitive for most wound clinics. The challenge is to develop sensors which offer similar information but at real-time, lower cost and better portability. The use of chemiresistors, such as conducting polymer arrays made from substituted pyrroles, constitutes a relatively cheap approach to gas analysis but suffers from sensitivity problems, sensor response time and interference by humidity (Bailey et al. 2008). Despite these disadvantages, a clinical trial by Parry et al. (1995) involving 21 patients using an Odormapper instrument housing 20 semiconducting polymer elements was able to instantly detect \( \beta \)-haemolytic streptococci in infected wounds.

Metal oxide sensors made from a large selection of metal types are an attractive low-cost alternative which are based on the principle of temperature dependent resistance modulation of thin semi-conducting metal oxide films by
the adsorption and desorption of gases (Wang et al. 2010). Tin and indium oxides in an array format have been used to analyse wound swabs later identified using standard microbiological assays to be infected with *E. coli*, *Ps. aeruginosa* and *S. aureus* (Setkus et al. 2006). Processing of the electrical output from the array format generated two-dimensional multi-layered plots that were matched to the type of bacteria by simple visual inspection. However, this study was carried out in a closed laboratory system with synthetic atmosphere to reduce unpredictable sensor response; reproduction of this environment is clearly a hurdle to translating this technique to the clinic.

The problems associated with using metal oxide sensors in the presence of background gases was addressed in a study by Feng et al. (2011). They used wavelet analysis of fourteen metal oxide sensors and one electrochemical sensor, all commercially sourced, to extract wound information in mice from the background of the natural and rich odor of the animals. This algorithmic approach to extracting fine distinctions between channels does not overcome the inherent poor discriminating power of the narrowly tuned metal oxide sensors. In future it may be possible to develop biomimetic sensors inspired by odorant receptors in insects which have superior independent sampling of broader regions of odorant space for better discrimination of volatiles compared with currently available sensors (Berna et al. 2009).

### 3.4 Temperature as an indicator for infection

Calor (heat) is an established marker of infection in wounds and may be used as an early predictor of chronicity before any obvious changes to the appearance of the wound are observed (Nakagami et al. 2010). It is relatively easy and cheap to measure temperature of skin and wounds using hand-held infrared thermometers. In a study of at-risk patients it was shown that infrared thermometers have the sensitivity to detect early signs of ulceration when used to measure temperature gradients between feet where differences of $>4^\circ F$ were used as the trigger point (Armstrong et al. 2007). While temperature is a very useful parameter to measure when monitoring wound healing there has been limited research into incorporating temperature sensors into wound dressings.
One approach reported is to use wireless temperature sensors based on the resistivity of carbon nanotubes coupled to a transponder to predict bedsores and inflammation (Matzeu et al. 2011). These sensors were tested on healthy volunteers but no reports have been made where they were used on patients.

Wireless technology may be useful in a clinic but ideally the wound dressing would incorporate some sort of stand-alone indicator of temperature, for example colorimetric sensors for measuring temperature. Color changing fibers with sensitivity of +/− 0.5 °C developed specifically for detecting infection have been patented by the group of Cranston but no research papers exist on this work (Mestrovic 2009). Many other types of low-cost colorimetric temperature sensors have been developed which could also be applied to wound management such as stimulus-responsive polymers attached to pSi in order to generate temperature and also pH sensors (Sciacca et al. 2011; Segal et al. 2007; Wu and Sailor 2009). For example, Vasani et al. (2011) employed surface-initiated atom transfer radical polymerization to graft a thermo-responsive polymer, poly(N-isopropylacrylamide) (PNIPAM), from pSi films producing a stimulus-responsive inorganic-organic composite material. The optical properties of this material changed significantly and reversibly when changing the temperature around the lower critical solution temperature (LCST) of the PNIPAM component.

Microfabrication of single crystal silicon nanomembrane diodes has been used by Kim et al. (2012) to develop electronic temperature sensors with resolution of 0.2 °C. An additional feature of the sensors is that they can also deliver heat to the tissue through a gold micro-heater to promote healing. Their sensors are intended for use in sutures but could equally be incorporated into dressings.

4. Sensors for detection of pH changes

4.1 Importance of pH

All biochemical processes in the body, including wound healing, are influenced by pH. Normally the pH of healthy skin is slightly acidic, in the range of pH 4-6, but when it is damaged this acidic milieu is disturbed as the body’s internal pH of 7.4 is exposed. In circumstances where the wound is acute, the pH follows a
relatively simple pathway through an acidic inflammation stage followed by a more basic granulation step before re-establishing in the pH 4-6 range during re-epithelization (Figure 4a). The pH of chronic wounds is substantially more complex. The somewhat simplified plot of wound pH in chronic wounds in Figure 4b (Schneider et al. 2007) suggests the pH oscillates between pH 7-8 as the wound fails to heal, however, there is evidence that the real situation is far more complex than this. Shi et al. (2011) found in a survey of the literature that pH values measured using electrodes designed for wounds or pH paper ranged from 5.4 up to 9.0 and that an increase in pH could be attributed to bacterial colonization. Srinivasan and Madhadevan (2010) have shown that a bacterial bioburden may not always necessarily result in an increase in pH as it is dependent on the primary energy source, at least for *E. coli*. Moreover there is anecdotal evidence that the composition (biofilm or planktonic), type of bacteria, anaerobic or aerobic conditions may influence pH so great caution should be exercised when using pH alone to diagnose wound status.

Despite the conflicting information on wound pH, its importance to the biochemical processes integral to wound healing means that pH is a marker of both diagnostic and theranostic interest. Consequently, several research groups have developed dressings which incorporate pH sensitive materials which are reviewed in the following section.

4.2 pH sensors

One of the simplest and most cost effective methods for determining pH is by using indicator dyes, which absorb different wavelengths of visible light depending on the pH. The challenges when using dyes incorporated into wound dressings is they must not leach from the dressing and they also have to be sensitive to the pH range encountered in wounds. Trupp et al. (2010) and Mohr et al. (2008) used the approach of synthesizing a series of hydroxyl-substituted azobenzene derivatives as indicator dyes for optically monitoring pH between 6-10 (Trupp et al. 2010) or 3-12 (Mohr et al. 2008). They tailored the sensitivity of the dyes by the substituent *para* or *ortho* to the hydroxyl group. The dyes were then immobilized on cellulose films using their pendent vinylsulfonyl groups. To
protect the cellulose from mechanical and swelling forces and to prevent shrivelling, it was laminated onto polyethylene terephthalate films. These laminates exhibited good reversibility and could be patterned into arrays, meaning a pH map of a wound may be possible. This technology has been used to assemble prototypes which turn from yellow to purple between pH values of 6.5 and 8.5 (www.technewsdaily.com/1454-expressive-bandage-displays-infectious-spread-.html)

Sridhar and Takahata (2009) have developed a microfabricated wireless pH monitor intended to be imbedded into a wound dressing to continuously track pH. The device uses a pair of wire coils, which sandwich a pH-sensitive hydrogel. As the hydrogel swells and deswells with pH, the distance separating the two planar coils changes which results in a change of inductance (Figure 4c). They observed a linear response in terms of inductance change with coil separation distance and changes in frequency over the pH range 2-7 when using poly(vinyl alcohol)-poly(acrylic acid) as the pH-sensitive hydrogel between the coils. The authors acknowledge that changes in moisture content may also change the reading and suggest using a pH-insensitive hydrogel in tandem to compensate for this. Interestingly, they also used a network-spectrum analyser with antenna to allow for wireless measurement of the coil gap, and hence pH. This approach may be valuable in cases where the smart dressing is covered by a compression bandage, provided of course that the bandage does not exert any force on the sensor.

A different approach to measuring pH of wound fluid has been reported by Phair et al. (2011) who exploited the presence of substantial amounts of endogenous uric acid in wound exudate to indirectly measure pH based on the oxidation potential change in uric acid with pH. Uric acid is the final breakdown product of purine catabolism and is present at concentrations >100 µmol L\(^{-1}\) in wound fluid (James et al. 2003). Their device features a laminated screen-printed planar electrode design with a cellulose filter paper wick for bringing exudate to the sensor. Importantly, their device contains no expensive components and is therefore disposable. The sensor was shown to be responsive to a wide range of test solutions ranging in pH from 3.7 to 8.0. When tested against blood from a healthy volunteer the measured pH matched the expected pH of 7.4, although
signal quality diminished over time as the uric acid was depleted because of oxidation and fouling of the electrode. This type of sensor would be unsuitable for incorporation into a dressing for continuous monitoring but may be useful as a POC dip-stick theranostic.

5. **Moisture**

Wound moisture levels are known to be critical to healing (Winter 1962; Wu et al. 1995) – too much moisture can result in maceration while too little can lead to the wound drying out (Banks et al. 1997). This key finding by Winter in the 1960s has led to a proliferation of dressing materials designed specifically to control the moisture content including films, hydrocolloids, foams, alginates and hydrogels (Queen et al. 1987; Queen et al. 2004). McColl et al. (2007) have developed an electrical impedance sensor which allows for real-time monitoring of moisture in an array format so a map of the wound can be obtained. Their sensor takes advantage of the ionic nature of wound fluid and quantifies fluid volume using alternating current of varying frequency and measures the impedance across Ag/AgCl electrodes insulated with a silicone elastomer inserted into a range of commercially available dressings. By inserting eight pairs of independent electrodes into the dressings, maps of hydration could be obtained. The system was tested *in vitro* with a simulated wound bed made from a Teflon mold with a liquid flow channel and syringe pump-controlled injectors to control delivery of an aqueous NaCl/CaCl₂ solution at four points below a wound dressing. The dressings were weighed periodically to obtain a relationship between change in impedance and loss of moisture. Impedance greater than 200 $\Omega$ indicated dehydration of the dressing and drying of the contact surface. Of the dressings examined, the polyurethane foams lost water rapidly while the polyurethane films retained moisture, presumably by occlusion which is not surprising as the film dressing is designed for lightly exuding wounds.

6. **Spectroscopy**
Vibrational spectroscopy is a technique that may be useful in the monitoring of wounds and although it is not strictly a sensor technique it is worth reviewing the state-of-the-art with respect to wounds as it may inspire sensor-type approaches in future. Hand-held portable spectrometers are now available making it feasible for a clinic to have one in their inventory (Erickson and Godavarty 2009). Monitoring of deoxy- and oxy-hemoglobin has been achieved using near infrared (NIR) spectroscopy initially in diabetic rat wound models (Weingarten et al. 2006; Weingarten et al. 2008) and recently in small human trials (Weingarten et al. 2010). NIR spectroscopy has been used to monitor oxygen concentration of hemoglobin in a number of other medical applications (Erickson and Godavarty 2009) based on the ability to distinguish between deoxy- and oxy-hemoglobin by virtue of their different absorbances. This may be exploited for use in monitoring wounds where it may be used as a reflection of impaired blood flow. Indeed, NIR spectroscopy has been used to distinguish between diabetic and non-diabetic wounds in rats (Weingarten et al. 2006). In a human study subsurface oxy-hemoglobin concentration was measured using a non-invasive NIR fibre-optic probe and found that hemoglobin concentration remained elevated in non-healing wounds. This was attributed to greater blood vessel ingrowth when compared with normally healing wounds which experience degradation and reabsorption of vessels in the late stage of healing when angiogenesis ceases (Weingarten et al. 2010). Significantly, the NIR technique offered early warning of non-healing wounds where some wounds which appeared to be healing based on traditional metrics were identified as having elevated haemoglobin by NIR.

7. Wound imaging

Imaging of whole wounds has the potential to offer the clinician much greater information than single-target diagnostics. In fact, one of the most basic and yet powerful tools in any wound clinic is visual observation of the site. Unfortunately, this approach is only useful if the observer has seen enough wounds, followed their progression and can confidently use this knowledge to inform their management program. Appreciably, this accumulation of experience
may take many years. Imaging and the dissemination of the images, therefore, has the ability to enable clinicians with a powerful, informative and non-invasive means to examine and quantify wound parameters. It further provides the potential to generate extensive databases of wound images that will be valuable for research, diagnostic and educational purposes.

Wound area is a key parameter used to monitoring healing and is often measured by tracing of the wound using transparent film or simply measuring with a ruler. Both these methods are subjective, time-consuming and marginally invasive. Commonly used alternatives, including ultrasound and digital photography, are useful methods for recording an image of the wound and allow for post-visitation analysis but suffer from color calibration for photography and identification of wound edges which can be difficult with complex wound topography. Papazoglou et al. (2010) have developed an advanced method for determining wound area using an algorithm that can detect and recognize a wound from a digital photograph. They used a standard low-cost point-and-shoot camera equipped with a polarizing filter to reduce light reflection. Consistency in lighting was achieved by photographing in the dark and relying on only the flash of the camera to provide a light source. The images were recorded as standard JPEG mode files and imported into a MATLAB program which identified a wound area and a non-wound area using grey scale images derived from the red, blue and green channels, as well as other color spaces with combinations of threshold and pixel-based color comparing segmentation methods (Figure 5a). They note that when there is clear color contrast it is easy to accurately and reproducibly measure the wound area. However, when the wound bed is inflamed, unclean, or if there is poor color contrast then repeatability is reduced.

The use of digital photography need not be constrained to recording of visible light. The ubiquity of cheap digital cameras may be the key to wound imaging when used in tandem with sensor films and ratiometric imaging. Meier et al. (2011) used a digital camera fitted with a 405 nm LED excitation ring to capture a 2-dimensional image of pH and partial pressure of oxygen (pO₂) within wounds. This work is based on luminescence life-time imaging sensors developed previously by the same group based on polystyrene-co-acrylonitrile
particles loaded with palladium(II)-porphyrin complexes, which are sensors for oxygen (Schreml et al. 2011b), or FITC as a pH-dependent luminophore (Schreml et al. 2011a). The mechanism for oxygen sensing is based on the oxygen-dependent quenching of the luminescence lifetime for the immobilized porphyrin. When a thin polyurethane film containing particles loaded with the $pO_2$ and pH sensors and a reference diphenylanthracene dye were placed on acute and chronic wounds, an image map showing $pO_2$ and pH was obtained using a digital camera with the 405 nm LED excitation (Figure 5b). This method works by separating the red, green and blue (RGB) channels of the camera and recording the output from different probes onto each channel. The emission of the Pt (II) probe, which is dependent on $pO_2$, and the luminescence of the FITC, which is a probe for pH, are recorded on the red and green channels, respectively, then referenced to diphenylanthracene dye in the blue channel. In the oxygen and pH maps (Figure 5b) of the chronic wound, the upper left corner is an area of low oxygen concentration and high pH, possibly indicative of inflammation which is absent in the acute wound.

The ability to measure $pO_2$ quickly and easily may make this technique useful in other POC diagnostics, such as an alternative to transcutaneous oxometry measurements (TCOM). This technique is routinely used on patients undergoing hyperbaric oxygen therapy for treatment of chronic wounds to confirm that oxygen is present in blood and plasma as a screening tool and predictor of therapy outcomes (Grolman et al. 2001). TCOM uses a heated patch on the skin to enhance oxygen diffusion to the surface where it is then measured. The sensor developed by Meier et al. may offer a smaller, cheaper alternative to traditional TCOM instruments, although these authors do not discuss this. In their paper, Meier et al. (2011) do, however, suggest their technology may also be used to probe temperature and to record videos of wounds.

8. Conclusions and Future Directions

Since the invention of the blood oxygen sensor in the 1960s by Clarke and Lyons (1962), biosensors have become a ubiquitous part of the research landscape. Two of the most successful and recognizable technologies which have gone on to
be commercially available are the blood glucose monitor for diabetics and the hCG hormone pregnancy test, but there are other sensor technologies now beginning to permeate the home diagnostics market targeted at variety of health conditions (Lee 2008). With the technology for measuring certain metabolites, proteins, gases and temperature now mature it is inevitable that these will make the transition into wound sensors as either disposable dip-stick type devices or as continuous monitoring tools in dressings.

The need for sensors for wound healing stems from the complexity of not just the dynamic wound healing biochemistry but also the management of wounds from a clinical perspective. There is strong anecdotal evidence that an important challenge in wound management is in the diversity of experience of the clinicians themselves, many of whom do not specialize in wound management. Sensors specially designed to detect key wound parameters have the potential to add objectivity to the practice of wound management. For example, there are indications that dressings are regularly being disturbed unnecessarily by premature renewal because the dressing looks full of exudate whereas the wound bed is still clean. A wound dressing with a built in moisture or infection sensor may aid in this decision-making.

The number of and sophistication of sensors is ever increasing but what is apparent through this review of the literature on sensors specifically focussed on wound healing, is that there aren’t actually very many identified targets being exploited, at least in the open literature, despite the long list of potential markers in Table 1. To date, the only wound markers targeted for sensor applications have been: i) infection (including odor and temperature), ii) pH, iii) oxygen iv) uric acid, v) hemoglobin and iv) broad spectrum protease activity (Systagenix’s Woundcheck™ product). Beyond this, reliable and specific targets have yet to be identified. Searches for biomarkers in wound fluid using proteomic approaches have been successful in finding differing distributions of specific proteins between acute healing and chronic non-healing wounds (Eming et al. 2010), which may be useful for future sensor development. However, it has been noted that a single protein approach may be unrealistic due to the complex balance between the proteins and their respective inhibitors (Wyffels et al. 2010).
Many of the sensors in this review (summarized in Table 2), although intended for use on wounds, are still in the early preclinical stages of development. The challenges of using sensors in real wound environments should not be underestimated since hundreds of different proteins are present at a range of concentrations (Eming et al. 2010) across pH levels as low as 5.4 and as high as 9.0 (Shi et al. 2011) within highly heterogeneous wounds. Continuous monitoring in-dressing sensors must be immune to protein fouling and cross-talk if they are to operate effectively. It may be that disposable dip-stick POC sensors are a more realistic option for many of the sensors discussed in this review. It is also important that many of the sensor outputs discussed should not be used in isolation. An example is pH, where an acidic or basic wound environment could be normal or abnormal depending on the progression of healing or state of infection.

The two types of sensors – diagnostic and theranostic, offer different information to the wound manager. Diagnostic outputs would be intended to inform what action is needed, for example, that the wound is infected and requires anti-microbial treatment. Theranostic output, conversely, for example, pH or presence of particular proteins, may be much more valuable in the study of wound healing especially if linked to databases (similar to the Proteomics Discovery Pipeline). The pooled data could then be linked to healing outcomes to further inform the practice of wound management.

Based on some of the leading edge research found in the literature reviewed here and some artistic licence we have drawn what we think the wound dressing of the future may conceivably look like (Figure 6). This dressing of the future incorporates a map of the wound using some of the imaging technology discussed as well as indicators for infection and pH and the time since the last dressing change. This is a view of how a dressing may look in 5-10 years but it’s conceivable that some of the current leading-edge sensor technology, such as stimuli responsive hologram images, wearable sensors and biocompatible photonics, such as silk waveguides will play a part in future concepts (Horgan et al. 2006; Kim et al. 2011; Parker et al. 2009).

Perhaps the most important outcome of this research is the impact it will have on patients. While it’s likely the clinicians will largely decide which sensor
technologies become accepted, it should be remembered that the technology should not be designed simply to make their job easier but to actually improve patient outcomes. Any sensor technology used in isolation will also be sure to fail and a holistic approach needs to be taken including patient nutrition and mental well-being.

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Conflicts
The authors have no conflict of interest.
Figure Captions

Figure 1: Current commercial products for wound management. Clockwise from top – Acticoat silver dressing (Acticoat (wound.smith-nephew.com), Mepilex moisture control dressing (http://www.molnlycke.com), Woundcheck™ Protease Status diagnostic (http://www.systagenix.com/our-products/lets-test).

Figure 2: (A) Polypropylene fabric impregnated with vesicles containing carboxyfluorescein under low UV intensity light in the presence of different bacteria. (B) The mechanism for bursting of the vesicles by bacteria releasing the fluorescent dye or an antimicrobial agent. (i) vesicle prior to rupture, (ii) toxins from bacteria lyse vesicle wall, (iii) contents of vesicle released. Adapted from Zhou et al. (2010) and Zhou et al. (2011).

Figure 3: a. Photoluminescence spectra (675 to 900 nm) of a porous silicon microcavity biosensor in the presence and absence of bacterial cell lysates. Blue spectra: sensor alone following derivatization with TWTCP and glycine methyl ester. Red spectra: sensor photoluminescence following incubation with cell lysates from Gram- (+) bacteria (B. subtilis, left) or Gram-(-) bacteria (E. coli, right). The green spectra in each case are the difference between spectra obtained in the presence and absence of bacterial cell lysates. Reproduced, with permission, from Chan et al. (2001). b. Detection of MMP-2 on a partially dried chip. (i) An optical image of the chip. (ii) Spectra of the reflected light taken from the spots that have been loaded with (from bottom to top) blank control, 0.1, 1, 10, 100, and 1000 ng/mL MMP-2 samples. Spectra are offset along the y axis for clarity. Reproduced with permission, from Gao et al. (2008).

Figure 4: a. pH of acute wounds, b. pH of chronic wounds. Images taken from Schneider et al. (2007). c. pH sensitive gel between inductance coils for continuous in situ pH monitoring (Sridhar and Takahata (2009)).

Figure 5: a. animal and human wounds traced manually (black line) and using image analysis (green, white, yellow lines) developed by Papazoglou et al. (2010). Scale bar=1cm b. Left: A digital camera fitted with a 405nm-LED ring light and an emission filter for photographing wounds covered in a sensor film. Right: visible light pictures, pO2 and pH maps comparing acute and chronic wounds. Image adapted from Meier et al. (2011).

Figure 6: The future of wound dressings? Our impression of a hypothetical wound dressing incorporating a color map of the wound, pH readout, infection feedback and indicator displaying if the dressing needs changing.
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Fig 2

**a.**

- Graph 1: Intensity vs. Wavelength (nm) for control and E. coli.
  - Control: Peaks at 675, 775, 875 nm.
  - E. coli: Peaks at 675, 775, 875 nm.

- Graph 2: Intensity vs. Wavelength (nm) for P. aeruginosa and S. aureus.
  - P. aeruginosa: Peaks at 675, 775, 875 nm.
  - S. aureus: Peaks at 675, 775, 875 nm.

**b.**

- Graph 1: Intensity vs. Wavelength (nm) for Blank control and MMP-2 concentrations in ng/ml.
  - Blank control: Peaks at 675, 775, 875 nm.
  - MMP-2 concentrations: Peaks at 675, 775, 875 nm.

Fig 3
Fig 4

(a) pH values of acute wounds
(b) pH values of chronic wounds

(c) Interconnect to backside coil & capacitor.

Folded flex circuit
Hydrogel
Flexural hinge

Wireless link
Breathable protection film

Fig 5

a. animal human

b. acute wound chronic wound

$\Delta pH$ $\Delta pO_2$ pH

4.5 4.5

0 150 75 50
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