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Proteomics in chronic wound research: Potentials in healing and health

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Abbreviations: ABPI, Ankle to brachial pressure indexing; **bFGF,** basic fibroblast growth factor; **CWF**, Chronic wound fluid; **DIGE**, Difference gel electrophoresis; **ICPL,** Isotope coded protein labels; **FDA**, Food and drug association; **iTRAQ**, Isobaric tags for relative and absolute quantitation; **MMP**, Matrix metalloproteases; **PUSH**, Pressure ulcer scale of healing; **TNF- α,** Tumour necrosis factor-α; **V.A.C.,** Vacuum assisted closure

Abstract

Chronic wounds, such as venous and diabetic leg ulcers, represent a significant health and financial burden to individuals and healthcare systems. In worst case scenarios this condition may require the amputation of an affected limb, with significant impact on patient quality of life and health. Presently there are no clinical biochemical analyses used in the diagnosis and management of this condition; moreover few biochemical therapies are accessible to patients. This presents a significant challenge in the efficient and efficacious treatment of chronic wounds by medical practitioners. A number of protein-centric investigations have analysed the wound environment and implicated a suite of molecular species predicted to be involved in the initiation or perpetuation of the condition. However, comprehensive proteomic investigation is yet to be engaged in the analysis of chronic wounds for the identification of molecular diagnostic/prognostic markers of healing or therapeutic targets. This review examines clinical chronic wound research and recommends a path towards proteomic investigation for the discovery of medically significant targets. Additionally, the supplementary documents associated with this review provide the first comprehensive summary of protein-centric, small molecule and elemental analyses in clinical chronic wound research.

1 Introduction

A chronic or non-healing wound is defined as any wound which takes a long time to heal (> 3 months), fails to heal by the use of conventional medical or surgical means, or recurs [1]. They predominantly comprise venous and/or arterial leg ulcers, diabetic foot ulcers and pressure ulcers. In the USA chronic wounds affect approximately 2% of the population and cost an estimated US$20 billion per year [2]. This rate is expected to be comparable in Australia and would equate to almost half a million Australians suffering from a chronic wound in 2009. The prevalence of chronic wounds is greatest among older people with an estimated 25% of all residents of aged care facilities suffering from a wound or multiple wounds [3]. Recent reports indicate that treatment and care of these wounds is around 3% of total health care expenditure in developed countries. In Australia, this equates to $2.6 billion in direct health care costs [4].

Beyond the direct costs of wound care, significant non-fiscal personal, social and quality of life costs exist. This lifestyle burden is potentially significantly greater than the fiscal burden and pervades all aspects of the economic and social fabric of developed countries. Chronic wounds require anywhere from several months to years to heal completely and in some cases, remain unhealed for decades. During this time patients experience severe emotional and physical stress, reduced mobility and limited productivity [5]. Patients with wounds require high levels of care with anecdotal reports from community health providers suggesting up to 50% of nursing time is spent caring for and treating chronic wounds.

In severe non-healing lower extremity wounds, where all available therapeutic interventions have been exhausted, amputation of the affected limb is necessary. Globally, diabetic ulcers

are the cause of 70% of all lower limb amputations, with one lower limb lost to diabetes every 30 seconds. In the Australian context this equates to more than 3000 lower leg amputations per annum [6], with similar rates of amputations associated with non-healing arterial ulcers. Following amputation, an estimated 50% of patients will die within 5 years, while the remainder often suffer poor quality of life, limited capacity to work and require significant investment in rehabilitation and ongoing care [7].

Clearly this health problem is set to amplify if innovative research is not engaged to improve the ability to diagnose, treat and manage chronic wounds. While numerous protein-centric studies have been reported in the quest for greater understanding of the chronic wound environment (e.g. [8-11]), proteomic approaches have been all but neglected. This review examines the current state of clinical chronic wound practice and research, and recommends a path towards proteomic investigation citing the potential for this approach to impact on health and healing.

2 The state of play: Clinical diagnostics, therapy and prognostics in chronic wound care

Diagnostic, therapeutic and prognostic technologies are the core of many clinical disease management strategies. These clinical approaches are underpinned by standard biochemical techniques for the efficacious treatment in most clinical presentations. This section outlines the current status of wound management and the opportunities for progress.

2.1 Diagnostics

Diagnosis of chronic wounds relies primarily on the classical clinical assessment of the presented wound [12]. Assessment criteria include analysis of the wound location, exudates, pain, location, oedema, lipodermosclerosis (fibrotic skin) and adjacent hyper-pigmentation. These factors in conjunction with a detailed clinical history form the basis of diagnosis. Venous and arterial etiology can then be inferred with the assessment of circulation in the lower limbs through sphygmomanometry and ankle to brachial pressure indexing (ABPI), whereby ABPI thresholds dictate venous and arterial state [13]. Doppler ultra-sonography is also commonly used in the determination of venous state [14]. In the case of diabetic and pressure ulcers, clinical history in conjunction with the assessment described above should be sufficient to determine wound derivation. Though work towards genetic [15] and biochemical [16, 17] profiling for diagnostics is reported, molecular markers are yet to be used in clinical practice for the diagnosis of chronic wounds [18].

2.2 Therapy

Several treatments are available to patients with chronic wounds. Aspects of wound care common to all ulcer types include debridement (removing adjacent necrotic tissue), infection prevention, moisture balance and treatment of co-morbidities (e.g. diabetes, oedema). In addition to this core regime, treatments are allocated to specific wound etiologies. Common treatments include compression bandaging therapy for venous leg ulcers [19, 20], vascular surgery for arterial ulcers [21], hyperbaric oxygen therapy in the treatment of diabetic foot ulcers [22] and removal of pressure/friction in pressure wounds [23]. Surgical intervention, in

the form of skin grafts and flaps, is also commonly engaged where conventional non-invasive treatments fail [24].

More recently oral inflammatory inhibitors [25] and products impregnated with growth factors [26] and other wound-modulating molecules have been introduced. Presently, recombinant platelet derived growth factor is the only Food and Drug Administration (FDA)-approved drug for the topical treatment of diabetic wounds and pressure ulcers [27, 28], while other growth factor treatments show promise [29]. These include vitronectin:growth factor complexes [30], which are currently undergoing clinical trial in Canada and Australia, and an epidermal growth factor-based formulation for diabetic foot ulcers [31]. Basic fibroblast growth factor is also in use in Japan as a topical therapeutic for the treatment of ulceration with scleroderma [32].

Other treatments include vacuum-assisted closure [33], topical hyperbaric oxygen therapy [34], acoustic therapy [35] and light therapy [36]. A good summary of other major approaches to chronic wound treatment can be found in [37].

2.3 Prognostics

As with chronic wound diagnostics, biochemical strategies are not yet employed in prognostic testing of chronic wounds [38]. Prognostic tools in chronic wound practice are derived from the scoring of physical clinical data. These tools are best described as wound classification systems which allow prediction of healing given the establishment of a temporal trend. Scoring systems for this end include the Pressure Ulcer Scale of Healing (PUSH) score [39], University of Texas score [40] and M.A.I.D. (M: presence of multiple ulcerations, A: wound

area, I: palpable pedal pulses, D: ulcer duration) score [41], which use a combination of wound area, circulation, exudate, oedema, duration and granulation tissue for calculation. While classification systems are unquestionably important in clinical practice, the use of multiple systems and the possibility for subjective assessment provides strong argument for a unified quantitative approach to chronic wound prognostics. As with many other conditions, the availability of such quantitative approaches requires considerable research and development.

3 Chronic wound research: Approaches to clinical sampling and analysis

3.1 Sample sources and collection

Samples for chronic wound research originate from two sources: tissue biopsies and the proximal fluid bathing the wound site (chronic wound fluid; CWF). Both have advantages and shortcomings which are discussed in a recent review [42]. While there is no general consensus as to an ideal sample for biomarker discovery, it may be assumed that a non-invasive approach would be best in a clinical setting. However, tissue biopsies may be necessary to ensure markers are sufficiently abundant for discovery, although they pose the risk of initiating a secondary chronic wound. Further, when it comes to controls, the gathering of proximal tissue from healthy patients may remain ethically challenging in some circumstances. Added argument for the use of CWF in wound research is gained from a study where differences in protein presentation in wound tissues showed significant variation between different sites of the same wound [43], potentially indicating superior consistency in the sampling of CWF.

3.2 Wound fluid

Chronic wound fluid is a unique bio-fluid enriched in wound-related proteins [18, 44, 45]. CWF is commonly gained through passive collection by trapping wound exudate behind an occlusive dressing. The fluid is then aspirated from behind the dressing and centrifuged to clarity [46]. Fluids have also been collected through alternate methods (see 5.2). The non-invasive methods of collection have made this sample type popular for the study of chronic wound healing. Research has analysed many CWF components and noted the fluid's cellular and extracellular modulating properties when the wound micro-environment has been re-created in the laboratory *in vitro* [47-54].

Control fluid samples are a contentious issue in chronic wound research. Studies have used serum [55-57], plasma [46, 58, 59], acute wound fluid [11, 60] and acute surgical fluid [61-63] for this purpose. Though no consensus has been reached, we contend that there is a strong rationale for patient matched serum or plasma. When biomarkers move to the clinic a patient's own serum will be an ideal control providing easy access and optimally matched sample-control conditions. Alternatively, regular assaying of patient CWF could provide for the establishment of quantitative prognostic trends [64]. Given large patient-to-patient variation, as illustrated in [64], the establishment of threshold values for clinical diagnosis may prove problematic.

3.3 Wound tissue

Wound tissues samples, like CWF, have been shown to be enriched in wound-related molecules (e.g. [8, 65, 66]). Tissues have been sourced from multiple sites within the greater

wound margin, commonly encompassing some of the surrounding intact epidermis. Specimens are either surgically removed or obtained by punch biopsy under local anaesthetic to be either snap-frozen or immediately formalin fixed. The invasive nature of this sampling procedure has, however, made research with clinical tissues less popular than CWF analyses.

As with control fluid, tissue controls in wound research are similarly variable. Authors have reported control tissue biopsies originating from sites both proximal [8] and distal [66] to ulcerated skin. We ourselves advocate the use of tissue proximal to the wound site, but beyond the boundary of obvious inflammation, fibrosis or hyper pigmentation. However, at risk of initiating a secondary chronic wound through biopsy, the gathering of proximal tissue controls may remain ethically challenging in some circumstances. To further complicate this argument, some suggestion has been put forth that creating an acute wound adjacent to a chronic wound site may in fact promote healing [67].

3.4 Analysis strategies

A number of strategies have been previously used in the identification and quantitation of proteins, small molecules and elements in clinical chronic wound research (Supplementary Tables 1-3). The primary source of absolute quantitation has been provided by enzyme-linked immuno-sorbent assays (ELISAs) for both tissue and CWF-based approaches, while Western blot analysis and immunohistochemistry have dominated identification strategies. Gelatine, casein and collagen zymographic techniques have also been important in implicating proteases, with chromogenic and photometric assays used to examine levels of proteolytic activity (see Supplementary Tables 1-3). Clinical and laboratory biochemical analyses are

also described for the quantitation and subsidiary identification of some proteins, small molecules and elements [56, 68].

To a far lesser degree, mass spectrometry (MS) and proteomic techniques, including 2-dimensional (2-D) gels and 2-D liquid chromatography, have been applied as analysis tools in chronic wound research [69, 70]. Given the rapid adaption of MS technologies to protein analysis in the study of disease, the lack of activity in this area would seem counterintuitive. However, several challenges surrounding the adaption of proteomic technologies to this condition (see 5.0) may offer an explanation in this regard.

3.5 The way forward

Diagnostics and prognostics of chronic wounds have progressed little in recent times despite the rapidly growing ageing population. By correctly identifying and quantifying holistic proteomic changes associated with the progression of disease, or its state, an abundance of information can be gleaned [71]. In this case it is anticipated that markers of healing or new therapeutic targets will be identified from temporal proteomic research [69]. Presently, only a single investigation has attempted to identify the protein components of wound fluid without bias [69]. Rather than antibody or zymography-based methods, this study reported for the first time the use of immuno-depletion, multidimensional chromatography, mass spectrometry and data interrogation techniques to separate, identify and catalogue the components of pooled CWF samples.

While limited in their approach, targeted studies are much more common throughout the literature and collectively are still able to provide valuable insights into the chronic wound environment.

4 A literature-derived proteome catalogue and biomarkers of wound healing

Although proteomic investigation of clinical wound samples is sparsely reported in the literature, a commendable body of protein-centric studies have facilitated important advances in understanding the wound environment.

4.1 A literature-derived proteome catalogue

As previously stated, the rationale for chronic wound fluid and tissue analysis is the potential for identification of therapeutic targets and/or prognostic and diagnostic markers of healing [44, 45, 63, 72]. Indeed, many investigations have been directed towards the evaluation of specific molecules hypothesized to be found in patient samples. The search for biomarkers can be easily assigned to three categories; protein analyses in CWF (Supplementary Table 1); small molecules and elemental analysis of CWF (Supplementary Table 2); and analyses of chronic wound tissues (Supplementary Table 3). This collection of published findings provides a somewhat limited, although available, literature-derived proteome catalogue as a reference for new and continuing investigators, as well as insight into the metabolic and electrolyte balance. Upon review, assembly of these data under their respective categories allows for analysis that highlights some additional information of interest.

4.2 Progress towards biomarkers and therapeutic targets

An initial analysis of the summarised data regarding the chronic wound-derived proteome emphasizes an overall focus towards chronic venous ulceration, which is not surprising given the dominance of this etiology. Duplication of targeted approaches is also evident in all three categories. Overall there has been a strong focus towards growth factors, cytokines and extracellular matrix proteins in previous research (Supplementary Tables 1 and 3). Again, this is unsurprising given the association of skin growth and regeneration (and associated imbalances) with these molecules. Duplicate investigation is particularly evident in the analysis of proteins in CWF, with elastase [43, 46, 55, 58, 61, 73-76], fibronectin [9, 11, 46, 55, 59, 60, 75, 77, 78], matrix metalloproteases (MMP)-2 [43, 46, 57, 61-63, 79-82] and -9 [43, 46, 62, 63, 79-81, 83, 84] actively investigated. These species in addition to others have been recommended as potential biomarkers or targets for therapeutic intervention. However, with the exception of elastase, MMP-2 and -9 [85, 86], few have progressed beyond recommendation.

The comprehensive investigation of MMP-9 in chronic wound healing has proven to be particularly fruitful. Building on a compelling body of work two recent papers have identified MMP-9 as a potential prognostic marker of healing in venous [79, 84] as well as diabetic [87] ulcers. This work precipitated the announcement of the development of a tool using MMP-9 levels for wound diagnostic purposes at the Third Congress of the World Union of Wound Healing Societies and the 7th National Australian Wound Management Association conference. In addition, this protease has been the focus of potential therapeutic intervention with the development of potential broad spectrum protease-modulating dressings [88] and other more targeted approaches [86].

The assessment of another member of the metalloproteases family, MMP-2, provides further evidence for the value of future proteomic investigation into clinical chronic wound healing. MMP-2 levels have been shown by a number of studies to be significantly increased over controls or to have a relationship to wound healing in wound fluid [43, 46, 57, 63, 80, 82] as well as wound tissue [79, 89, 90]. These data, in combination with the finding that tissue inhibitor of metalloproteases (TIMP)-1 is dysregulated in chronic wounds [10, 61, 79, 80, 83, 87] and that elastase activity and levels are also increased [43, 58, 61, 74, 91], led to the development of a protease modulating matrix, PROMOGRAN [85]. The matrix is composed of bovine collagen and oxidized regenerated cellulose and acts to absorb proteases and divert protease activity away from precious granulation tissue.

With the clinical presentation of self-sustaining inflammation in chronic wounds, the molecules responsible have also been the focus of fervent research (Supplementary Tables 1 and 3). As expected, a number of the inflammatory proteins targeted in previous investigations have been found to be increased over control concentrations [9, 92-95], or in some cases, related to the stage of chronic wound healing [95]. Following on from these results, research published in 2006 described the use of a recombinant chimeric protein for the ablation of tissue necrosis factor (TNF)-α activity in chronic wound fluid [25]. This paper showed the efficacy of this approach as a potential wound treatment. Subsequent work involved the use of infliximab, a TNF-α therapeutic antibody, in the treatment of chronic, therapy-resistant leg ulcers [96]. The paper demonstrated successful response in seven out of eight patients treated with the antibody therapy. Clinical trials are still required for the efficacy assessment of this approach in relation to traditional therapies.

The role of growth factors in wound healing has also been the subject of much investigative power. Dysregulation in the presentation of vascular endothelial growth factor [57, 65, 97], epithelial growth factor [98], hepatocyte growth factor [99], platelet-derived growth factor [82], transforming growth factor-β_1 [65, 81, 87, 93, 98, 100, 101], transforming growth factor-β_2 [100, 102] and insulin-like growth factor-1 [57, 103] has been reported from several laboratories. Significantly, the altered presentation of basic fibroblast growth factor (bFGF) and its statistical association with wound healing has been demonstrated [81, 104]. This work has now resulted in the development a bFGF-based therapeutic for skin ulceration in scleroderma for use in Japan [32] and again highlights the potential for new therapeutic targets to be discovered through proteomic investigation.

While substantial work towards examining chronic wound samples has been published, the selected approaches have provided only a limited number of targets (approximately 140 proteins and 20 small molecules/elements). Without detracting from the significant contribution of this work to the understanding of the chronic wound environment, recent proteomic characterisation of a comparable biological fluid [105] suggests a great deal of information is yet to be obtained.

Several recent relevant articles have advocated the use of clinical samples for biomarker discovery with the most recent directing readers towards protein profiling strategies [18]. Given this advocacy, the potential of proteomic investigation and the longevity of this research area, it begs the question: What's stalling the application of proteomics to clinical wound healing research?

5 Application of proteomics to chronic wound healing: Challenges and innovations

As with analysis of any clinical condition, the application of proteomics to wound research has its specific challenges which have hindered the progress and adaptation of this investigative approach to wound healing research. This section outlines some of those challenges and appropriate steps for progress.

5.1 Challenges of sample collection

The recruitment and collection of samples is the first obstacle in any clinical investigation. Even prior to patient recruitment the collection of clinical data and samples should be conducted in such a way as to protect the patient. Chronic wound tissue biopsies can therefore be ethically challenging, and impossible in the authors' own research experience, given the potential to initiate a secondary chronic wound. CWF is therefore the default sample type in some cases but can harbour its own drawbacks as outlined below.

Given ethical clearance, patient recruitment for chronic wound research can prove equally difficult. Many patients present with co-etiologies and co-morbidities [106], including local infection and biofilms [107], and many may be unable to participate fully due to illness or mortality. Anecdotally, these conditions can limit patient recruitment given suitable study inclusion criteria.

5.2 Challenges of clinical chronic wound samples

CWF is a highly variable clinical sample. Measurements of pH, protein concentration, volume and bacteriology have returned large ranges from a cross section of wound types. With regard

to proteomic investigation, volume and protein concentration are fundamental. In the authors' laboratories wound fluid concentrations have been determined to range from 0.05 – 50.5 mg/ml while volumes have varied from 0.02 – 2.5 ml. Similarly, total protein amounts have spanned 0.07 – 47 mg protein per sample. Such variability in these CWF parameters challenges the clinical proteomic investigator in terms of analysis methods and technique development.

Dynamic range is an issue well known in serum-based proteomics. Given the gross similarities evident between serum and wound fluid [108] and the presence of low abundant species, such as growth factors and cytokines, CWF components would appear to span a similar concentration range. Elevated proteases, leading to chronic wound-related protein degradation [11, 46, 55, 59], further contributes to the complexity of this sample.

Several collection techniques are also prevalent in the literature. These include: passive techniques such as direct aspiration from beneath occlusive dressings [61]; direct extraction from wound dressings [52]; hydrophilic absorption [83]; micro-dialysis [68, 109]; and the active technique, and by-product of vacuum-assisted closure (V.A.C.), negative pressure accumulation [110]. The variation in these techniques may arguably impair the comparison of studies from multiple laboratories and eliminate robust metadata analyses. Indeed, comparison of passive CWF accumulation in dressings and CWF actively accumulated by V.A.C. has shown large differences in the amount of exudate and protein obtained [110], suggesting that molecules of interest may be diluted to the detriment of proteomic investigation. As yet there have been no objective investigations into the efficiency and efficacy of the available CWF collection techniques. Ideally, samples should be collected

under comparable patient conditions with the least opportunity for irreversible adsorption, dilution, loss or degradation of components.

Clinical wound tissue also shares the challenge of dynamic range. As skin composition is dominated by multiple keratin proteins, the wound researcher is required to engage pre-fractionation or sophisticated multidimensional fractionation techniques. Also, as mentioned earlier, there is the irregular presentation of proteins from multiple sites within the same wound [43], suggesting that several sites may need to be biopsied which then raises ethical issues, as indicated in 5.1.

5.3 Innovative approaches and recommendations for progress

Multi-site recruitment and clinical sample collection is imperative for the progress of chronic wound research. In this regard, significant patient numbers which represent distinctive aetiologies can be obtained. Harmonization of collection, processing and storage methods between sites and laboratories may also prove useful if data are to be shared or comparisons drawn. Given the above criteria for optimal collection, aspiration of wound fluid which has been passively collected from beneath an occlusive dressing is the authors' recommendation for standardized collection. While the other techniques have their merits, none offers the same non-selectivity, or possibility to obtain a whole, undiluted sample. CWF should then be clarified by centrifugation as soon as possible and stored frozen at -80 °C until analysis. In our experience CWF samples should also undergo the least number of freeze-thaw cycles and not be left at ambient or elevated temperature for any extended period due to the visible degradation of CWF components when analysed by gel electrophoresis.

With regard to dynamic range, normalisation [111] or affinity depletion [112] technologies offer assistance. Current depletion strategies promise to selectively remove up to 20 of the most abundant proteins found in serum, plasma and related fluids [113]. Indeed, depletion technology has been shown to be adaptable to wound fluid samples [69], with the successful depletion of seven of the most abundant serum proteins and subsequent identification of several CWF proteins previously undescribed in this fluid. Caution in the application of this technology is necessary though as column binding capacities can be reduced, as stated by the manufacturers, if serum/plasma has originated from patients with an inflammatory condition (depletion-targeted components may be elevated to the point where they are not able to be retained using the recommended protocol). Dynamic range in wound tissue analysis may also be manageable with new technologies for high resolution separation such as p*I*-based liquid-phase fractionation [114].

Given the variable protein amounts available from small clinical samples it may be necessary to engage highly sensitive MS or antibody array [115] technologies. These array technologies are particularly attractive to the chronic wound researcher given the use of small sample amounts and the high specificity of the technology. Arrays can further be targeted to specific molecular functions, such as protease action, growth factors or structural proteins [116]. Alternatively, chemical labelling strategies where temporal samples are pooled prior to identification (e.g. iTRAQ, ICPL, ExacTag, DIGE) offer another approach to deal with sample scarcity. Multiple reaction monitoring may also prove useful in the validation of targets, or as a means to analyze large temporal/multi-patient sample sets, due to its enhanced selectivity and sensitivity and ability to schedule transitions [117, 118].

Innovation in the validation of chronic wound fluid targets may also offer valuable insights into the wound environment. The development of an *in situ* western blot technique, where a membrane is placed directly on the surface of a wound bed to maintain spatial information could be easily implemented. The membrane could then be probed for a target of interest, thereby identifying the region of the wound where that molecule is present and therefore the region best targeted for appropriate therapy.

6 Potential for proteomics to impact chronic wound practice

The identification of therapeutic targets and prognostic or diagnostic markers of healing has the potential to revolutionize chronic wound care. We believe that proteomic approaches in chronic wound research are the likely avenue for the discovery of wound healing-related molecules for the development of biochemical analyses and/or therapy development. Rapid and specific identification of the wound etiology will inform clinical decision making and ensure patients are correctly managed, while the development of advanced therapies will further reduce practitioner burden and decrease financial load on healthcare systems.

Indeed, as the economic and social burden of chronic wounds is set to increase due to the ageing of the population and a surge in associated underlying medical conditions, these innovations are desperately needed. Older people and those with chronic diseases, such as diabetes and vascular disease, are at greatest risk of developing a chronic wound. The Australian Bureau of Statistics projects that by 2050 over 25% of the population will be over 65 years of age, almost twice that in 2007 (13%). By 2031 it is predicted that 3 million Australians will be diabetic, with 20% of these patients being at risk of developing chronic wounds (http://www.aihw.gov.au/publications/index.cfm/title/10394).

Improving capacity to manage, treat and plan for chronic wounds is therefore essential for the future of health care systems world-wide. The projected increase in the prevalence of chronic wounds is also reflected in the size and growth of the advanced wound care products market, which in 2006 was estimated at US$2.6 billion with a growth rate of 12.3% per annum over 5 years to US$4.6 billion according to market research firm Piribo Ltd.

Despite the significant economic and social impact of chronic wounds, efforts to overcome this challenge have been limited in Australia and world-wide, with wounds typically managed as co-morbidities of other conditions and by clinicians with limited specialization or fragmented training.

7 Conclusion and perspective

Clinical chronic wound research has yet to venture beyond targeted strategies for the identification of molecules of medical interest. Proteomics offers a powerful technique for this purpose. Previous targeted approaches have identified significant differences between chronic wound samples and related controls eliciting the question of the yet undescribed whole proteomic changes to be identified. Several barriers have historically delayed the uptake of this approach in wound research; however, advancements in tools for the improvement of proteomic depth offer encouragement to move forward into this arena.

The collation of previous research findings and recommendations for research outlook described herein outline a path towards the discovery of molecules of medical interest in the diagnosis, treatment and management of chronic wounds. The uptake of antibody array

technologies in this quest is particularly attractive given issues with clinical sampling and their multiplexed and targeted nature. Chemical labelling techniques [119] and enrichment strategies [69] also offer avenues to negate issues of sample size and complexity which, like serum-based proteomics, appear to plague CWF research.

Given the current scope of chronic wound proteomics, any information gained through this approach will contribute to greater understanding of these collective conditions. The discovery of definitive diagnostic or prognostic markers, or novel therapeutic targets, will significantly improve what is a major and increasing health challenge. It remains now for the research community to take up this challenge for the improvement of health and healing.

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8. References

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