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Bioimpedance spectroscopy - can it be used as a tool for monitoring fluid shifts in burns?

Pippa Kenworthy
The University of Notre Dame Australia

Bioimpedance Spectroscopy – can it be used as a tool for monitoring fluid shifts in burns?

Pippa Kenworthy BSc (Physiotherapy)

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Declaration And Statement Of Contributors

This thesis contains published work and/or work prepared for publication, **which has been co-authored**. The bibliographical details of the work are presented for each study. The work involved in designing the studies described in this thesis was performed primarily by Pippa Kenworthy (candidate). The thesis outline and experimental design was planned and developed by the candidate, in consultation and assistance from Dr Tiffany Grisbrook, Dr William Gibson, Assoc Prof Dale Edgar and W. Prof. Fiona Wood (the candidate's supervisors).

All participant recruitment and management was carried out or facilitated by the candidate between December 2014 and February 2017. This was completed in association with the staff and patients of the Burn Unit and Medical Illustrations Department, initially at Royal Perth Hospital and then at Fiona Stanley Hospital (FSH) (as the burn service and unit transitioned to the new FSH February 2015). The Fiona Wood Foundation (Chevron Fellowship) has supported my clinical research time for the duration of the study.

In addition, the candidate was responsible for the data analysis with assistance from Mr Michael Phillips, biostatistician, Medical Research Fund of Royal Perth Hospital. The candidate drafted the original thesis, with Dr Tiffany Grisbrook, Dr William Gibson, Michael Phillips, Assoc Prof Dale Edgar and W. Prof. Fiona Wood providing feedback on drafts until the examinable version was finalised.

I declare that all of the material presented in this thesis is original.

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Pippa Kenworthy
Senior Physiotherapist
Burns Service of Western Australia
Fiona Stanley Hospital
P: +61 08 615 72926
E: pippa.kenworthy@health.wa.gov.au

Assoc Prof Dale Edgar, Head,
Burn Injury Research Node,
The University of Notre Dame
Australia

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Abstract

Large fluid shifts and oedema are features of burn injuries. Oedema hampers burn wound healing and is directly related to the size and depth of the burn. The degree of oedema in burns covers a broad spectrum: Minor burns cause localised or peripheral oedema, whilst major burns may result in a systemic inflammatory response which can be life threatening and necessitates formal fluid resuscitation. Acute burn fluid resuscitation is paramount in decreasing patient morbidity and mortality but can contribute to already large amounts of oedema. There is currently no single clinically applicable, non-invasive and accurate outcome measure to titrate fluid volumes in acute burns or monitor the effect of treatments on oedema (in minor and major burns). Bioimpedance spectroscopy (BIS) has emerged as a possible solution to these challenges. It can measure body fluid compartments and thus fluid volume changes over time providing a sensitive non-invasive device to estimate resuscitation requirements and oedema change and is emerging as a measure of wound healing. This series of studies therefore aimed to 1) address the potential barriers to use of BIS in the burns population, 2) determine if BIS provides an accurate measure of whole body/systemic fluid volume change and 3) localised burn wound oedema changes, as applied across the spectrum of burn severity, and 4) determine if BIS can monitor wound healing in minor burns.

The studies therefore investigated novel whole body and localised electrode positions in the presence of open and dressed wounds, using repeated measures over time in minor and major burns.

The key novel findings arising from the research series include: 1) alternate electrode placements are interchangeable with standardised placement for the measurement of whole body resistance, extracellular and total body fluid volumes in specified dressing conditions. Therefore BIS can be utilised to monitor changes in fluid shifts when wounds preclude the manufacturer's standard placement of electrodes in the presence of burn wounds, 2) BIS is a reliable method of monitoring fluid in any dressing condition and electrode position with no systematic bias indicated in both major and minor burns, 3) In both minor and major burns, BIS is a valid indicator of

net fluid shifts and oedema change, if dressing condition is adjusted for using the developed algorithms or calculator and 4) BIS resistance variables, R_0 and R_{inf}, can be used to monitor wound healing in minor limb burns as an adjunct to standard practice.

Publications And Abstracts

The following is a list of peer reviewed publications I have contributed to during the course of candidature. A total of six co-authored papers were prepared, submitted or published during the candidacy. The first four of these contribute directly to the thesis (in the order they appear).

Peer Reviewed Publications

1. **Kenworthy P**, Grisbrook TL, Phillips M, Wood FM, Gibson W, Edgar DW. Addressing the barriers to bioimpendance spectroscopy in major burns: alternate electrode placements. J Burn Care Res. 2017 Mar 15.
2. **Kenworthy P**, Grisbrook TL, Phillips M, Wood FM, Gibson W, Edgar DW. An objective measure for the assessment and management of fluid shifts in acute major burns. Accepted to Burns and Trauma, November 2017.
3. **Kenworthy P**, Grisbrook TL, Gittings P, Phillips M, Wood FM, Gibson W, Edgar DW. Bioimpedance spectroscopy: A technique to monitor interventions for swelling in minor burns. Burns. 2017 August 3. DOI: 10.1016/j.burns. 2017. 04.022
4. **Kenworthy P**, Grisbrook TL, Phillips M, Wood FM, Gibson W, Edgar DW. Monitoring wound healing in minor burns – a novel approach. Burns. 2017 August 4. DOI: http://dx.doi.org/10.1016/j.burns.2017.06.007
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1. **Kenworthy P**, Grisbrook TL, Gittings P, Phillips M, Wood FM, Gibson W, Edgar DW. A technique to monitor interventions for swelling in minor burns: A pilot

study. Bullet poster presentation. Proceedings of the Australian and New Zealand Burns Association, October 2015, Melbourne, Australia.

2. Grisbrook TL, **Kenworthy P**, Phillips M, Wood FM, Edgar DW. Nanaocrystalline silver dressings influence Bioimpedance Spectroscopy measurements in burns patients. Proceedings of the Australian and New Zealand Burns Association, October 2015, Melbourne, Australia
3. **Kenworthy P**, Grisbrook TL, Phillips M , Gittings P, Wood FM, Gibson W, Edgar DW. Confirmation of a novel localised measure for swelling in patients with acute burn wounds. Proceedings of IHR Health Research Symposium, December 2016, Perth, Australia.
4. **Kenworthy P**, Grisbrook TL, Phillips M, Gittings P, Wood FM, Gibson W, Edgar DW. Bioimpedance spectroscopy: A technique to monitor interventions for swelling in minor burns. Poster presentation. Proceedings of the British Burns Association, May 2017, London, United Kingdom

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List And Definitions Of Key Terms

Acticoat ™		Ionic silver antimicrobial burn and wound dressing (Smith & Nephew) (1)
Bioimpedance spectroscopy	BIS	A method used to assess body composition and allows for evaluation of specific body compartments and cell health such as fat free mass (FFM), inter-compartmental fluid volumes (extra and intra cellular fluid and total body fluid) and cell mass (2, 3). It uses a range of frequencies from 4-1000 kHz.
Bioimpedance Analysis	BIA	Like BIS it is a method used to assess body composition and allows for evaluation of specific body compartments and cell health. It is either a single frequency or multi-frequency method.
Body Cell Mass	BCM	Reflects the actively metabolizing cellular compartment. Indicated by ICF.
Burn Service of Western Australia	BSWA	Includes Fiona Stanley Hospital (FSH)
Extracellular fluid.	ECF	Fluid outside the cell consists of interstitial fluid (~13L) (dense connective tissue and bone), plasma (~3L) and transcellular fluid (~1L) (4).
Fluid resuscitation		Intravenous ± oral fluid given in the first 24-48 hours of moderate to large burn injury to maintain intravascular volumes (5)
Intracellular fluid	ICF	Fluid contained within the cell, has a high K^+ content (95%) as well as Mg^+, phosphates and protein (4)
Lean Body Mass	LBM	Body weight – fat mass
Net Fluid Shift		The difference between the input and output of fluids over a specified timeframe
Oedema		The fluid which traverses from the intravascular space into the extravascular space in response to tissue injury (6, 7). Otherwise known as swelling
Phase angle	PA	A measure of cell membrane vitality and prognostic indicator of malnutrition and disease. Calculated as the arc tangent of Xc/R and expressed in degrees (8).
Resistance at infinite frequency	R_{inf}	An index of TBF and used in the calculation of estimates of TBF (9).
Resistance at zero frequency	R_0	An index of ECF and used in the calculation of estimates of ECF (9).

Resistance of intracellular fluid	R_i	An index of ICF and used in the calculation of estimates of ICF (9).
Total body fluid	TBF	ECF + ICF. 56%-70% of the body consist of fluid, equivalent to 35-45 L in an average sized human being (4)
Total body surface area	TBSA	Expressed as a percentage

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As you can see I was rarely alone as I travelled down the research path. My fellow physiotherapy colleagues who were flexible and worked with me throughout the data collection process (and may have had an open ear at times to my statistical nightmares).

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Chapter 1 Introduction

Burns are one of the most traumatic injuries a patient can sustain often having a lifelong impact on a person's quality of function, both physically and mentally. Burn injury causes tissue damage, and a unique inflammatory response, which results in marked oedema. The inflammatory mediators released, such as prostaglandins, histamine and bradykinin, increase intravascular permeability and promote the passage of fluid into interstitial spaces causing local and systemic fluid shifts (1, 2). Excess oedema inhibits blood flow and reduces oxygen perfusion in vulnerable tissue, resulting in worsening of the burn wound (3). Immediate management and ongoing monitoring of oedema is therefore essential in limiting the severity of burn injury, especially in the first 48 – 72 hours (4).

Optimising emergency treatment of burns is paramount to achieve the best possible outcome. Fluid resuscitation is an important aspect of acute burns management in burns greater than 15-20% total body surface area (TBSA) and may be thought of as the cornerstone of burns early management and patient survival (5). Despite this, there has been limited innovation or progress in interventions in the area over the last 30 years (6). Initial fluid resuscitation volumes delivered are determined using formulas based on the patients weight and %TBSA (7, 8). Fluid volumes are monitored closely and titrated, most commonly, according to urine output (30 – 50ml/hour) (9). Administering acute fluid resuscitation volumes as closely aligned to those initially calculated and closely monitoring urine output is essential in the prevention of burn shock and other complications such as abdominal and peripheral compartment syndromes, kidney failure, pulmonary oedema and peripheral tissue oedema (10). Fluid resuscitation in the first twenty four hours post burns remains a complex task as the patient must receive sufficient fluid to prevent hypovolemia and ensure adequate tissue perfusion and blood supply to vital organs but it will also accentuate the oedema process (1, 11, 12). Both peripheral and systemic oedema can contribute to burn wound complications and delay wound healing. It therefore needs to be optimally managed (10, 13). To optimally manage oedema a reliable and accurate measurement device is firstly required.

Current methods of oedema or fluid shift assessment in burns are either invasive, time consuming, require an open wound or are an indirect measure. Fluid resuscitation volumes in moderate to large burns are initially determined using accepted formulas as guidelines e.g. Parkland formula (14, 15) and are then titrated using most commonly, urine output and haemodynamic observations such as oxygen saturation and blood pressure. Other objective measures used to guide fluid volume titration are: pulmonary artery catheterisation and transpulmonary thermodilution (provide right heart diagnostic information to rapidly determine hemodynamic pressures, cardiac output, and mixed venous blood sampling) and base deficit and lactate (16, 17). These are all indirect (and invasive) methods of measuring fluid volumes and attempts to normalise cardiac output and haematocrit in the first 48 hours of injury does not improve patient outcomes and may lead to over resuscitation (18). Fluid creep, the tendency to administer too much intravenous fluid is not uncommon (6).

The widely accepted methods for clinical monitoring of peripheral oedema are circumferential limb measures (CLM) and water displacement volumetry (WDV) (19). These are both confounded when wounds are dressed; pose a potential infection risk and WDV can be cumbersome. Limited progress in identification of clinically applicable oedema measures has contributed to the lack of emergent interventions for more proactive oedema removal (20). Thus, to guide improvements in fluid resuscitation and oedema management in the burn population, a non-invasive, easy to use, accurate assessment of oedema is required.

Bioimpedance spectroscopy (BIS) is a technique used to assess and monitor an individual's body composition, such as inter-compartmental fluid volumes, fat free mass and cell health (21, 22). It is an instrument frequently utilised in healthy populations, lymphoedema and more recently in other clinical populations such as dialysis patients (23, 24). There are many studies investigating its use as a method of assessing and monitoring malnutrition, fluid shifts in the critically ill and after surgery and as a prognostic tool in cancer (25-28). Bioimpedance spectroscopy is gaining popularity as method of assessment in the aforementioned areas as it is practical, rapid and has demonstrated sensitivity and reliability (29, 30).

The bioimpedance spectroscopy instrument applies a small alternating current into the body over a range of frequencies (4-1000 kHz), via electrodes, providing instantaneous measures of resistance (R) and reactance (capacitive resistance (Xc)). Resistance is the opposition to flow of an electric current and capacitance is the delay in the passage of current through the cell membranes and tissue interfaces. The flow and path of the electrical current is frequency (Hz) dependent (Figure 1.1). Resistances at zero and infinite frequencies (considered ideal measurement frequencies) are estimated utilising the Cole-Cole plot embedded in the BIS software, due the constraints of using a direct or very high frequency alternating current in humans (31). The resistance at zero (R_0) and infinite (R_{inf}) frequencies (32) are representative of extracellular fluid (ECF) and total body fluid (TBF) respectively. Resistance (R_i) of the intracellular fluid (ICF) is extrapolated using the other raw variable data. At low frequencies, the current cannot traverse the cell membrane and will only pass through the ECF, which surrounds the cells. At high frequencies (>50 kHz) the current will pass through the ECF and the cell membrane or intracellular compartment thus estimating TBF (33) (Figure 1.1). When these raw resistance variables are incorporated into predictive mixture theory equations (e.g. Hanai equation) embedded in the BIS software, fluid volumes (ECF, ICF, TBF) can be calculated (32). The raw resistance variables can also be utilised to monitor inter-compartmental fluid volume changes as body fluid behaves as resistive components and resistance is inversely proportional to fluid volume and therefore oedema (34, 35). Reactance is caused by the capacitance of the cell membrane and represents cell membrane mass and function. Another variable, phase angle (PA), is a measurement calculated from the relationship between R and Xc (36). It is a predictor of cell health, and has been shown to have potential in the ability to monitor wound healing and as a prognostic indicator of malnutrition and disease (30).

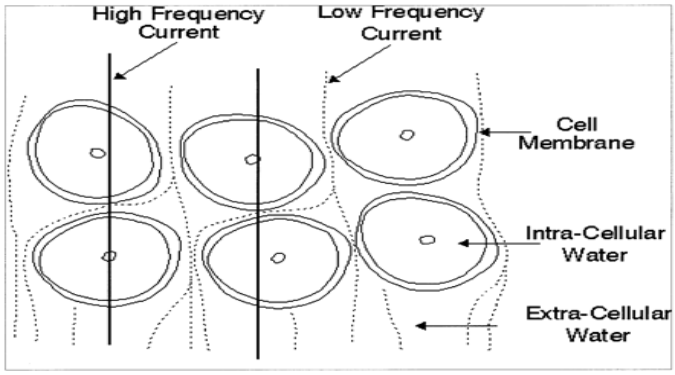

Figure 1.1: Current distribution in cell suspensions (37)

Bioimpedance can measure fluid shifts at a whole body level, with electrodes placed on the hands and feet (38). Fluid volume change and cell health of limbs or wounds can be measured simply by placing the electrodes either side of the segment to be measured. This is termed segmental or localised bioimpedance. It brings the field of measurement closer to the site of interest and is more sensitive to fluid volume changes compared to whole body measures (35).

To date there is limited literature investigating the use of bioimpedance in burns. Zdolsek (1998) found whole body bioimpedance analysis (BIA) was sensitive enough to determine the development of oedema (using TBF) and the effects of fluid resuscitation following burn injury (15-63% TBSA) (39). This was however a small and underpowered study (n=9). Another study by Miller et al (1999) found there was a significant positive correlation (r=0.958) between single frequency whole body BIA and the titrated water method of determining TBF in patients with severe burns (<23% TBSA range 23-65%). More recently, Edgar et al (2009) used whole body BIS to measure acute oedema shifts in human burn survivors in different dressing conditions (29). They concluded that BIS analysis is clinically applicable for the real time monitoring of whole body fluid shifts in patients with injuries less than 30% TBSA regardless of dressing conditions, but it was more reliable with no dressings than when dressings were in place. They did not however, explore the reason dressings affected the reliability of BIS variables. These researchers demonstrated BIS has the ability to monitor fluid changes in acute burns, however they did not confirm whether it is a valid measure of fluid shift to be able to clinically titrate fluid resuscitation volumes.

There are no studies in the literature, exploring BIS as a measure of localised limb oedema or wound healing in burn injured patients. However in muscle injuries, single frequency bioimpedance analysis (BIA) with localised electrode placement (sense and drive either side of the injury) was able to detect changes in oedema and cellular injury consistent with MRI imaging over time in the individual (41). In patients with wounds of varying aetiologies, localised single frequency bioimpedance variables R, Xc and PA were found to increase with re-epithelialisation of a wound, with modest decreases after wound debridement and greater decreases with methicillin-resistant staphylococcus aureus (MRSA) infection of a wound (30). The rate of change in the raw variables signalled the presence of infection before detection with laboratory methods (30). Measuring local wound and peripheral oedema practically and with ease in a clinical setting will guide improvements in pro-active oedema management and thus aid wound healing.

There are potential barriers to the use of BIS in burns such as open wounds and dressings. These may prevent the placement of standardised electrode positions and influence the BIS variable output. There is conflicting evidence regarding movement of standardised electrode positions and repeatability of BIS variables. The theory of equi-potentials, which are loci of points with the same electrical potential and perpendicular to the flow of the current, suggest movement of electrodes at points circumferentially will yield the same results as standard electrode placements (42). Movement proximally however, by one and two centimetres has been reported to change mean resistance values by 2% and 4% respectively, indicating BIS is a highly sensitive measure (43). Thus, the standardisation and accuracy of electrode placement is important to minimise BIS reproducibility errors between measures. Acute burn wounds, minor and major, will have a dressing in place at all times except at the time of dressing change. Further, commonly burn dressings are impregnated with silver ions or are water based (hydrocolloid). Considering resistance (opposition by a conductor; a type of material that allows the flow of electrical current in one or more directions) is proportional to the amount of fluid and ionic content of the fluid, it is not unrealistic to expect burn dressings may alter the BIS variable measures (resistance and calculated fluid volumes). Silver is a highly conductive material and will therefore likely decrease the measured BIS resistance. Hydrocolloid dressings will also be expected to affect the resistance measured due to

it being an ionic dressing. Edgar et al (2009), as previously mentioned, demonstrated BIS measurements were less sensitive in older dressings (>8 hours old) compared to when no dressings were in place (29). Moving forward, being able to utilise BIS when dressings are in place and/or when wounds prevent standardised electrode positioning would enhance the applicability of BIS clinically in burns and other clinical environments.

1.1 Statement Of The Problem

All burn injuries result in a cascade of inflammatory mediators and oedema. Oedema is detrimental to wound healing. However, minimal advance is observed in the interventions to control or reduce oedema volume in wounded tissue. The lack of advances may be attributed to a lack of accurate, clinically viable assessment of new methods. Whether it is a localised wound or systemic oedema there is no single non-invasive, real time, bedside measure of monitoring oedema change. It is evident in both the literature and clinical setting that oedema can lead to conversion of an acute burn wound, limb and abdominal compartment syndromes and slow healing. These negative sequelae of oedema can significantly impact an individual's physical function, scar quality, psychological recovery and even morbidity outcome. These adverse outcomes lead to increased medical costs, increased length of stay and an increased burden on the patient and family. There has been little advance in 1) the assessment and monitoring of burns resuscitation fluid monitoring and titration in the last forty years and 2) in the assessment and treatment of peripheral limb oedema. A major reason for this is due to the lack of user friendly, sensitive outcome measures. Bioimpedance spectroscopy is a method of oedema measurement and wound healing, which has merit in burns. It is worth investigating BIS in this challenging unique population where dressings and open wounds often hinder the use of the gold standard measures of oedema volume (WDV and CLM) (4, 19).

1.1.1 Aims Of Study Series

This research aims to assess whether BIS is a reliable and valid measure of fluid volume change, across the spectrum of burn severity. Secondly, it aims to address the barriers, such as wounds and dressings that may impede the use of BIS in this

environment. It will attempt to provide solutions to overcome these potential barriers, through investigation of i) novel electrode placements and ii) the effect of dressings on BIS variable outputs. Thirdly, the study aims to establish whether BIS can be used to monitor local wound healing.

1.1.2 Significance Of Study Series

This series of studies will aim to provide a solution for real time, practical assessment and monitoring of fluid volume change and wound healing in burn patients, providing solutions to real clinical problems and therefore help guide improved clinical care of the patient.

The basis of this research is to provide a reliable and valid measure of oedema change so it can i) guide future intervention studies to progress proactive oedema management and ii) improve oedema assessment clinically to allow application and adjustment of the current best practice management strategies in the burn population. Thus significantly impacting patient outcome and recovery following a burn, as "every intervention from the point of injury influences the outcome after burn" (4).

It is anticipated the findings of this research will be applicable to all staff responsible for the wound care in burn patients, from nursing and medical staff to allied health professionals. All of these team members will benefit from the findings of this research, as all members are involved in aspects of oedema control, prevention and wound healing management. The findings hope to drive clinician behavioural change with respect to positive changes in proactive oedema management and care, through the use of BIS in standard clinical practice. By delivering the outcomes of the study to burns clinicians and translating the use of BIS into clinical practice, it will re-iterate and reinforce the importance of oedema management in the care of the burn patient. It also has the potential to reduce the cost to the health system.

1.2 Thesis Outline

The context for the study, the research problem and its significance are presented in the introduction and literature review and they communicate the steps taken to address the questions posed. This is followed by a series of studies exploring the

challenges to the use of BIS in burns and establishment of its methodological utility and concluding with a synthesis of the results and discussion. Both studies investigate the use of BIS in acute burns, across the spectrum of severity as major burns cause a systemic inflammatory response and minor a localised inflammatory response. Each class of burn also have their own, similar but unique, potential barriers to the use of BIS.

The first study relates to the assessment of whole body fluid shifts, using BIS, in acute burns requiring fluid resuscitation. It is disclosed as two papers; (i) addresses the issue of wounds to the placement of BIS electrodes and (ii) presents the reliability and validity of BIS and factors that influence BIS variables.

The second study is also presented as two papers. The first of these addresses the barriers to the application of BIS and the reliability and validity of novel localised BIS in the assessment of minor limb burn oedema. The second presents localised BIS as a method of monitoring wound healing. The thesis therefore consists of four separate inter-related papers.

There are aspects of each of the studies that are similar due to the nature of the burn environment and the similar aims addressed across the burns spectrum. The references are presented at the end of each studies manuscript. A synthesis of the results and discussion concludes the thesis.

This series of studies was conducted at Fiona Stanley Hospital (FSH), the tertiary hospital for the Burn Service of Western Australia (BSWA), a state-wide service. Understanding the current clinical practices and model of care of the service will provide context and insight to the research methodology for the study series. The BSWA utilises the modified Parkland formula to instigate initial intravenous fluid resuscitation (2 ml/kg/hr) in burns greater than 15% TBSA, or as deemed clinically necessary. Fluid volumes are monitored and titrated according to urine output (0.5 ml/kg/hr) and haemodynamic monitoring. Limb oedema is most commonly assessed subjectively and with CLM. Oedema management is integral to clinical practice and a priority of all treatments. The BSWA employs a multi-disciplinary approach in ongoing oedema management practices. The most common oedema management principles applied are: education; elevation using positioning devices such as lower

limb wedge cushions and axilla arm boards; low stretch compression using - Coban 3M™ (Critical & Chronic Care Solutions, New South Wales, Australia) self adherent wrap, tubular-form (Sutherland Medical Pty.Ltd., Victoria, Australia) and oedema gloves; cardiovascular fitness exercise such as walking, exercise bike and arm ergometer; active range of motion to enhance lymph flow and strength/resisted exercise. Wounds in the first 48 hours of injury are managed with an antimicrobial, silver impregnated dressing (Acticoat™) as it has been shown to be effective against most common strains of wound pathogens; decreases pain levels; reduces infection rates; and is cost effective (44). Dressing choice after this period is dependent on the status of the wound as decided by the clinical specialist.

1.2.1 Study One: Addressing The Barriers To Bioimpedance Spectroscopy In Major Burns: Alternate Electrode Placements

The aim of this study was to:

- Determine whether alternate electrode configurations for whole body and limb segmental BIS outputs were comparable to standardised electrode configurations in moderate to large size burns across different dressing conditions

It was hypothesised that:

- Whole body and limb segmental alternate electrode positions will provide comparable BIS variable output, raw and predicted, to standard electrode positions

Conclusion: Whole body resistance variables and extracellular fluid can monitor changes in fluid shifts with alternate electrode placements where wounds preclude standardised placement in both an open wound and Acticoat™ dressing. It was also apparent the Acticoat™ dressing exaggerated the differences between the standard and alternate electrode positions but also between the open wound and Acticoat™ dressing condition.

1.2.2 Study Two: An Objective Measure For The Assessment And Management Of Fluid Shifts In Acute Major Burns

The aims of this study were to:

- Examine the reliability of BIS with respect to dressing condition and electrode position.
- Establish the effect of Acticoat^{TM} dressings on BIS variable outputs
- Determine the validity of whole body BIS in the presence of major burns

It was hypothesised that:

- BIS will be reliable in any dressing and electrode position
- Acticoat^{TM} dressings used in the first 48 hours of burn injury in the Burn Service of Western Australia (BSWA) will reduce BIS variable outputs
- BIS raw resistance variables will decrease and predicted fluid volumes will increase with increasing fluid shift

Conclusion: Whole body bioimpedance is a valid indicator of net fluid shifts, if dressing condition is adjusted for.

1.2.3 Study Three: Bioimpedance Spectroscopy: A Technique To Monitor Interventions For Swelling In Minor Burns

The aims of this study were to:

- Examine the reliability and validity of the BIS technique for the measurement of localised burn wound oedema with respect to electrode position and dressing condition.

It was hypothesised that:

- Bioimpedance resistance variables, R_0, R_i, R_{inf} will increase as limb volumes decrease.

Conclusion: BIS is a sensitive, reliable and valid technique that may be used clinically to monitor localised changes in burn wound oedema.

1.2.4 Study Four: Monitoring Wound Healing In Minor Burns – A Novel Approach

The aim of this study was to:

- Determine whether the BIS technique is a valid measure of wound healing

It was hypothesised that:

- BIS resistance and phase angle will increase with burn wound healing

Conclusion: BIS is a technique, which has the potential to monitor the wound healing process of a minor acute burn.

1.2.5 Synthesis Of Results And Conclusions

This final chapter draws the results of the individual studies together, providing an integrated discussion of the major findings, study limitations and future research and gives an encompassing conclusion of the entire research.

1.3 References

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Chapter 2 Review Of The Literature

This literature review provides an overview of oedema production after a burn injury, its potential impacts in the burn wound environment and why it is important to have a reliable and valid bedside oedema assessment tool. The pathophysiology of burn wound healing and oedema is firstly discussed. It then outlines factors contributing to burn severity and oedema volumes. Followed then by a discussion of current burn oedema or fluid monitoring outcome measures and their limitations. Bioimpedance spectroscopy is subsequently introduced. A brief overview of BIS properties and its potential uses in the burns environment is provided.

2.1 Wound Healing

Inflammation is the body's normal response to injury. It is a complex process of vascular and cellular responses protecting the body against infection. Normal healing of an acute wound occurs in a timely and orderly sequential manner (1). Factors impacting the normal pathway to healing include injury severity, age, co-morbidities and ethnicity (2). The longer a wound takes to heal the greater the risk of infection and hypertrophic scarring.

The ability for the skin to heal largely depends on the extent of the injury (2). Skin can be simply divided into three layers (Figure 2.1) (3). Understanding the structure of these three layers and the burn depth can provide invaluable information on expected wound healing times and necessary medical interventions.

i. Epidermis: outer most superficial layer of skin. It is composed of epithelial tissue. The dermal epidermal junction, the interface between the epidermis and dermis, attach the two layers to each other and is a key to epithelial repair. The epidermis also influences the dermis with regards to structural remodelling, re-innervation and vascularisation (3).
ii. Dermis: has two layers, the papillary and reticular layers. The upper, papillary layer, contains a thin arrangement of collagen fibres and the lower, reticular layer, is thicker and made of thick collagen fibres arranged parallel to the

surface of the skin. The dermis consists of oil and sweat glands and hair follicles. The types of tissue are: collagen, elastic tissue and reticular fibres.

iii. Subcutaneous tissue: a layer of fat and connective tissue that houses larger blood vessels and nerves. This layer is important in the regulation of skin and body temperature.

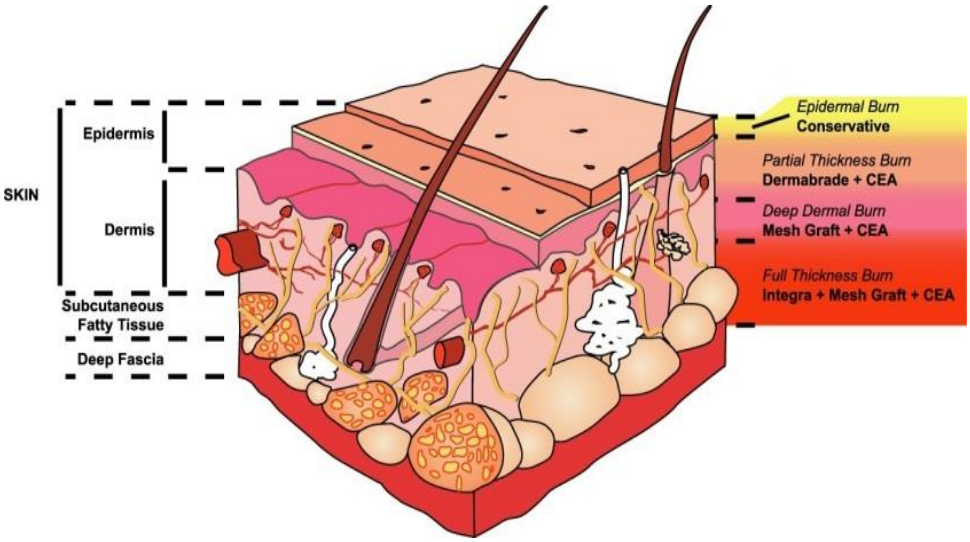

Figure 2.1*:* Cross section of burn depth and skin layers (reproduced with permission F. Wood)

Superficial burns (involving epidermis and papillary dermis) will regenerate epithelium from sufficient unburned epithelial appendages, allowing spontaneous healing with minimal scarring. Deep partial and full thickness burns (deep dermis to subcutaneous tissue) are slow to heal with resultant unstable skin and hypertrophic scarring (4, 5).

2.1.1 Factors Affecting Wound Healing

Multiple factors can impair healing and they exist at a local wound and systemic level. The following are a common but not exhaustive list of patient factors affecting healing:

- Pre-existing disease (e.g. peripheral vascular disease, diabetes) and increasing age. Both result in an altered inflammatory response compared to normal and are predisposing factors to oedema formation (6).

- Alcoholism, obesity and smoking increase the risk of vascular, heart and lung disease. These diseases commonly cause affect the structure and function of the vessels which can interfere with oxygen supply (2).
- Ethnicity, pre-existing nutrition and stress also impact wound healing (2).

Additionally, injury severity, presence of infection and oedema all contribute to the healing capacity of a wound (7).

A burn injury results in a hypermetabolic response and increased catabolism of protein. In severe injuries the body can be catabolic during wound healing greater than 12 months after injury (8, 9). The body requires sufficient and generally extra nutrients to promote healing and sustain hypermetabolism. A high caloric and nutritious diet is therefore needed. Further detail here is beyond the scope of this project but it is necessary to understand factors influencing a healing wound.

Burn injuries are highly susceptible to infections due to loss of skin integrity and reduced cell mediated immunity (10). The presence of infection will slow wound healing and leads to altered fluid dynamics and extravasation of oedema (6). Burn patients are susceptible to infection due to the removal of skin, the protective barrier; general immunosuppression; surgical intervention; prolonged hospital impatient stay; and the environment of injury (11). It is the main cause of mortality and morbidity for burn injured patients (10). Wound colonisation with microorganisms delay wound healing, increase graft loss and increase risk of systemic infection (1, 12). It is vital that wound management is optimum and infection control procedures (e.g. sterilisation and/or cleaning of equipment, hand hygiene) are adhered to (13).

Burn wound dressings are therefore important and provide a variety of benefits. They protect the wound from further trauma or infection by providing a barrier to infection, provide comfort and pain relief, and promote healing (14). There are number of different dressings and choice is dependent on various factors: the extent of injury, stage of healing, amount of exudate, patients intact skin integrity, presence of infection, position of injury, surgical intervention. The BSWA protocol is to apply Acticoat^{TM} dressings to all burns for the first forty-eight hours of injury, then change as appropriate according to the wound condition.

2.2 Burn Wound Response

Oedema, a natural inflammatory response to trauma, is a normal part of the healing process. This response however is exaggerated in a burn injury causing excessive tissue fluid deposition, both locally and systemically (15, 16).

2.2.1 Zones Of Injury

The burn wound is described in three zones of tissue injury (Figure 2.2). 1) The irreversible zone of necrosis – the extent of which is directly related to the temperature and duration of exposure and is irreversible due to coagulation of constituent proteins (16). 2) The zone of stasis - characterized by decreased tissue perfusion. In the first 48-72 hours it can be salvaged through timely and appropriate intervention. In this area, excess oedema can further decrease tissue perfusion converting salvageable to necrotic tissue, a process known as burn wound conversion (17). 3) The outermost zone of hyperaemia – an area of tissue with increased perfusion, which surrounds the zone of stasis. It is invariably oedematous recoverable tissue (17). Oedema, a natural response to trauma through inflammation, is a normal part of the healing process. However, excessive oedema can result in increased tissue losses, slow wound healing, exacerbate tissue scarring, limit function and at worst increase mortality (15, 18).

2.2.2 Burn Wound Conversion

Burn wound conversion is an important phenomenon in the treatment of thermal injury as burn wound depth may be a significant determinant of morbidity and mortality. It is also clinically significant because as the degree of burn advances it increases the likelihood of hypertrophic scarring, contractures, need for surgical excision and grafting, wound infection, sepsis and shock (19).

Burn wound conversion is a term for the dynamic process resulting in increased tissue losses and wound deepening. It is caused by many factors both local and systemic such as excess oedema, changes in blood flow, excess inflammation and inflammatory mediators, infection and chronic medical illnesses (e.g. diabetes, vascular disease) (20). Excess oedema limits the exchange of vital nutrients

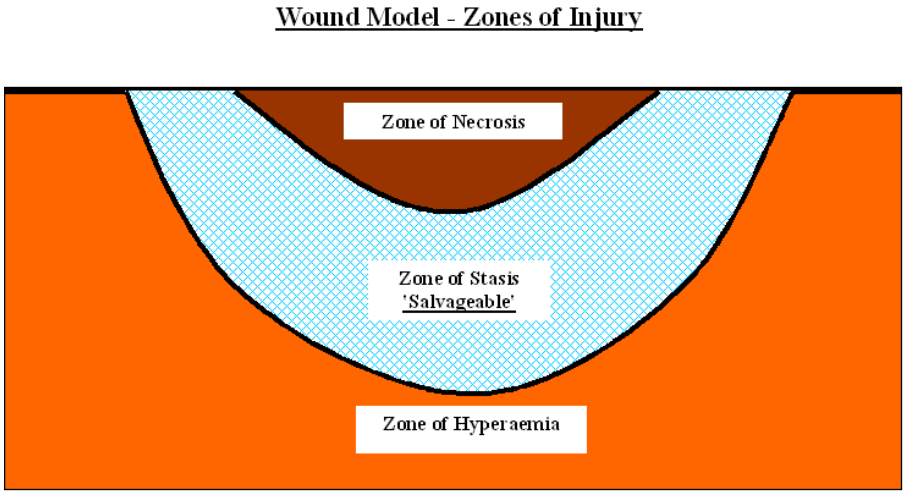

Figure 2.2: Diagrammatic representation of the zones of injury (reproduced with permission, D.Edgar)

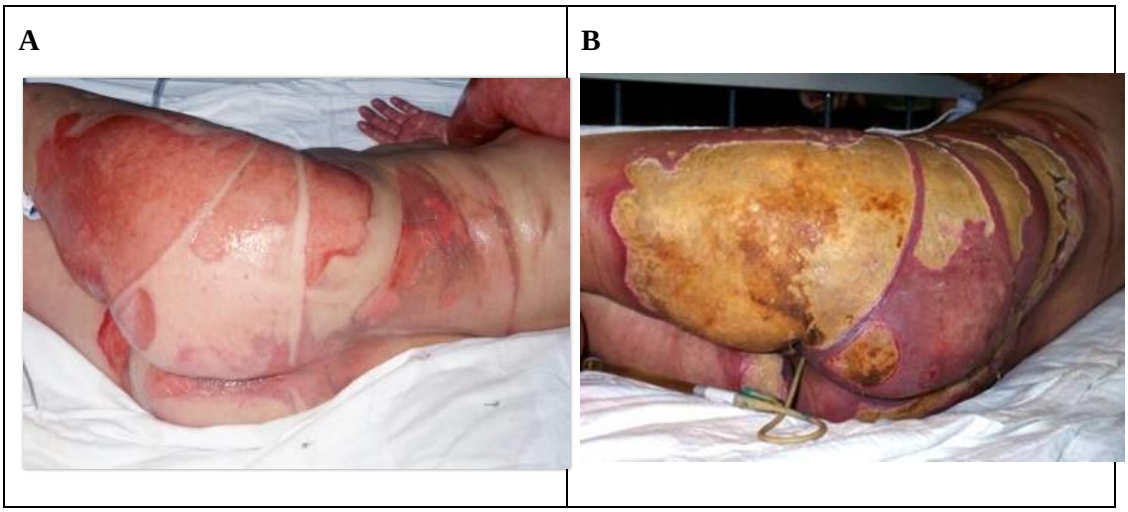

Figure 2.3: Demonstration of burn wound conversion in a scald injury. Mid dermal (A) to full thickness (B).

(including oxygen), between the circulation and the damaged areas compromising vulnerable tissues (21). Conversion is commonly seen in the subacute phase (three-five days) where burns initially assessed as superficial-mid dermal thickness progress to deep partial or full thickness burns (Figure 2.3). Thus, timely removal of oedema is paramount in limiting the risk of burn wound conversion. Also, as the TBSA of the burn increases so too does the risk of wound progression (17). Limiting the degree of both local and systemic oedema through optimal control of fluid resuscitation in major burns, elevation, movement, compression where appropriate and using

appropriate wound dressings, maintaining nutritional status and timely surgery can reduce the risk of burn wound depth.

2.2.3 Burn Pathophysiology

The pathophysiology of the microvascular changes post-burn is quite complex. It is known that when burnt the damaged tissues release chemical mediators, such as histamine, prostaglandins and oxidants (all cells altered from the burn injury are capable of releasing oxidants), which can damage the capillary membrane and increase capillary permeability (22). This allows leakage of fluids, plasma proteins and electrolytes from the intravascular space into the extravascular space (or tissues) causing immediate localised oedema, and in burns greater than 15-20% TBSA, systemic oedema (swelling in non-injured tissues and the lungs) (23). This is known as the 'vascular leakage syndrome' noted to last for ~24 hours after burn. The syndrome is life threatening and immediate medical attention is recommended as it can lead to burn shock, a unique phenomenon, which is a combination of distributive, hypovolemic and cardiogenic shock (22). Fluid resuscitation is necessary to maintain circulating blood volume and blood supply to vital organs, but it also contributes to oedema in the tissues, particularly during the 'leaky blood vessel' period.

Unavoidable local oedema and large scale fluid shifts are caused by disruption of collagen cross linking destroying the integrity of the osmotic and hydrostatic pressure gradients (24). There is also worsening fluid regulation and systemic inflammatory responses due to cell membrane damage from the influx of inflammatory mediators exacerbating abnormal cell to cell permeability.

These fluid shifts and resultant micro-thrombi in vessels can exacerbate hypoperfusion (or inhibition of blood flow) in vulnerable tissue, specifically in the zone of stasis and hyperaemia i.e. inadequate oxygen perfusion can increase the zone of necrosis thus worsening the burn wound (25). Limiting excess oedema is therefore important as it can reduce healing time via optimal blood flow and oxygen to the wound thus positively benefiting burn survivors.

2.3 Contributing Factors To Acute Burn Oedema

Acute burn oedema formation and resolution is related to the severity of the injury including factors such as the depth and size of burn, immediate first aid management and fluid resuscitation.

2.3.1 Burn Depth

Burn depth is defined according to the layer of skin damaged i.e. epidermis, dermis, subcutaneous fat and can be divided into five categories of increasing depth: epidermal, superficial dermal, mid dermal, deep dermal and full thickness (Table 2.1) (26).

Burn depth affects the volume and location of oedema. Superficial and mid dermal burns have a greater local, immediate oedematous response than full thickness burns (22, 27). Excess ECF (oedema) can be persistent in deeper burns due to the disrupted integrity of capillaries and increased capillary leak. The capacity for oedema to be reabsorbed into the vascular system and be carried away by the lymphatics in a timely fashion as it is in normal wound healing, is therefore reduced (28).

In partial thickness burns, oedema located mainly in the dermis, increases in the first few hours and then gradually reabsorbs over three-four days due to the preserved lymphatic system (17, 22). Oedema in deep or full thickness burns increases at a slower rate and over a longer period due to damaged dermal vascular and lymphatic channels with reports of peak levels at 18 hours after-injury (29). Twenty five percent of oedema in deep burns is still present at one week.

In pigs inflicted with minor burns, Papp et al (2006) found superficial burns had increased water content of the whole dermis and subcutaneous fat at eight hours after burn; partial thickness burns had a greater water content in the whole dermis still at 24 and 72 hours after burn; Full thickness burns presented with significantly less water content in the upper dermis at 24 hours and was associated with necrosis of the tissue layer (30). All burns had higher tissue water content in the subcutaneous fat compared to non burned areas. In sheep inflicted with burns, oedema was located in the surrounding dermis and subfascial tissue for all burn depths and in underlying adipose and muscle in full thickness burns of sheep (31). It has been demonstrated

the distribution and the rate of occurrence of oedema and the capacity of the body to reabsorb oedema is related to burn depth. It is just one component of burn injury supporting the importance of early oedema management and monitoring.

Table 2.1: Burn depth characteristics.

Depth	Colour	Blisters	Capillary Refill	Sensation	Healing
Epidermal	Red	No	Present	Present	Yes (3-7 days)
Superficial Dermal	Pale pink	Small	Present	Painful	Yes (7-10 days with minimal dressings)
Mid-dermal	Dark pink	Present	Sluggish	+/-	Usually (should heal within 14 days)
Deep Dermal	Blotchy red	+/-	Absent	Absent	No (generally needs surgical intervention)
Full thickness	White	No	Absent	Absent	No (generally needs surgical intervention)

[Table adjusted from the Emergency management of Severe Burns course manual 2013] (26).

2.3.2 Total Body Surface Area

Total body surface area influences the volume of oedema production due to increased tissue damage increasing the rate or volume of vascular permeability (22). Increasing TBSA is also associated with an increased risk of burn wound conversion and is an indication of overall burn severity (16, 17). Generally minor burns < 10-15% TBSA result in localised burn wound oedema and major burns > 20-25% TBSA induce a systemic reaction with significant ‘vascular-leak’ or hyper-permeability of

the capillaries in the first 24-48 hours after surgery (32). Research has estimated that in large burns up to 50% of oedema volume is in non-burn areas (22). Total body surface area is one of the main considerations in the determining fluid resuscitation volumes in large burns and is determined most commonly using the 'rule of nines' (Figure 2.4).

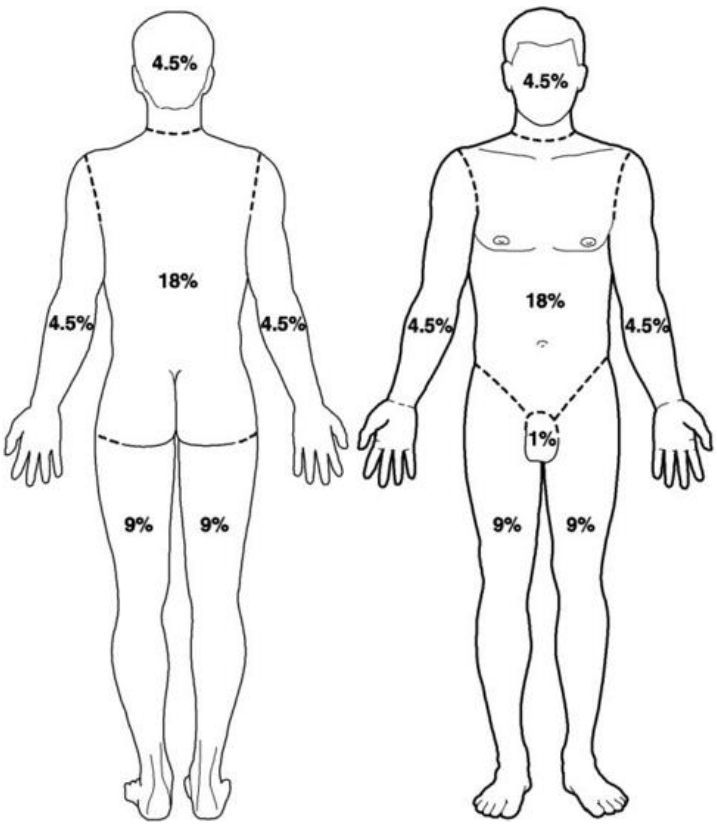

Figure 2.4: Rule of nines for estimation of burn severity in adults (33)

2.3.3 Inhalation Injury

As the size of the burn increases so does the risk of inhalation injury and it will occur in two-thirds of patients with greater than 70% TBSA burn injury (5). Upper airway oedema can occur rapidly in patients with smoke inhalation and a sizable burn. Intubation should not be delayed. Patients considered at risk of inhalation injury should be assessed and monitored with arterial blood gases, chest x-rays and pulse oximetry. If the equipment is available, monitoring of end tidal carbon dioxide using capnometry or capnography can provide useful respiratory status information. Fiberoptic laryngoscopy and bronchoscopy can assess the extent of airway injury

(34). These are indirect measures of oedema but contribute to the overall picture of injury severity.

The scope of this thesis is investigation of whole body and localised limb wound oedema and not distinguishing airway oedema. However, it is known major burns are at risk of pulmonary oedema due to vascular leak in the initial stage of injury (16). After reviewing the literature, Saffle et al (2007) reported fluid resuscitation requirements of patients with an inhalation injury was greater than those patients without an inhalation injury (from 35% to 65% greater) (35). This was independent of the type of resuscitation fluid delivered.

2.3.4 First Aid

Recommended immediate first aid management is 20 minutes of cool running water (15° - 18°C), occurring up to three hours after the injury has occurred at the burn site whilst keeping the patient warm (36). It significantly lessens the impact of the injury through reducing scar and infection and the need for surgery (37).

2.3.5 Fluid Resuscitation

A major goal of the initial management of burn injuries is to replace ECF loss proportional to %TBSA of the burn. This is in the form of intravenous fluids in large burns. Optimal fluid resuscitation is important to: maintain the circulating blood volume; supply blood to vital organs; help prevent local impaired wound perfusion through maintenance of perfusion pressures to maximally oxygenate the injured and non-injured tissues; and systemically to restore intracellular and intravascular fluid volumes thus improving cellular respiration and increasing tissue perfusion (17, 20, 22).

Resuscitation itself is a source of fluid that leaks into the tissues and contributes to the oedema (38). It is therefore important to be as precise as possible, giving the minimal volumes of fluid required to achieve vital organ perfusion and limiting contribution to oedema. There are a number of formulas used to guide initial volumes of fluid required for adequate resuscitation in partial to full thickness burns

exceeding 15-20% TBSA (39-41) but the Parkland formula developed by Baxter and Shires's over 40 years ago is most widely used (23, 24, 38).

The Parkland formula calculates total fluid requirements using lactate ringer's (crystalloid) solution in the first 24 hours from injury as 4mL/kg/%TBSA and the current Emergency Management of Severe Burns formula is 3-4mL/kg/%TBSA (42, 43). The total volume is divided in half and half the fluid given intravenously over the first eight hours following the burn and the remaining volume over the next 16 hours. In addition, two litres of background fluid is administered. For a 70 kg person, with a 20% TBSA burn, this can equate to a total of 6200 – 7600 ml of resuscitation fluid delivered in 24 hours. The host of formulas utilised in the literature are almost all based on weight and burn size and use various combinations of fluids.

2.3.5.1 Fluid Creep

It has been demonstrated in recent times that over-resuscitation or delivery of fluid volumes in excess of those predicted is a frequent occurrence, a phenomenon known as 'fluid creep' (43-45). It can negatively impact a patient's outcome and contributes to the volume of oedema caused by the acute burn injury. The reasons for this fluid creep remain unclear. Saffle et al (2007) post review of the literature suggested clinicians are instinctively adopting a 'more is better' approach with less stringent adherence to guideline formulas as a decrease in mortality is being seen with aggressive fluid resuscitation (35). In 2000, Engrav et al conducted a survey of 28 burn centres in the USA and found 58% of patients received greater than 4ml/kg/%TBSA of fluid (46).

The use of opioid drugs is another primary cause thought to contribute to this phenomenon. Opioids given in high doses for pain relief are known to cause hypovolemia, as they have significant effects on the cardiovascular system (contribute to vasodilation) thus increasing fluid requirements (46, 47). Current pain control interventions have improved and more opioids are given now than in the 1970's (24) contributing to the higher incidence of over resuscitation. In 2004 a study by Friedrich et al compared a group of patients treated at a Washington burn centre from 1975-1978 to a similar group of patients (%TBSA, sex, age) treated in 2000 and found the latter group received significantly more fluid per %TBSA and

significantly more opioid agonists than the 1970's group. They concluded that opioid dosage correlated positively with fluid requirements (48).

Other proposed reasons behind 'fluid creep' have been identified and may include: patients with inhalation injuries, electrical burns, delayed resuscitation, other traumatic injuries, pre-existing disease and nutritional status and previous alcohol or drug abuse. These patients are most likely seen to require additional fluid to maintain end organ perfusion (24, 35, 49).

Inexperienced clinicians may also contribute to increased resuscitation volumes by making substantial errors in estimating burn area and depth, which can result in significant under or over calculation of fluid requirements. Despite these known influences the accuracy of Parkland formula has not been challenged by these reports, rather it has emphasized the necessity of monitoring patients carefully and adjusting fluid infusions based on patients' response (35, 44). It has proposed the need for valid and sensitive monitoring devices (22, 35).

Fluid creep and its prevention are imperative due to the increase risk of adverse outcomes. Well documented side effects of over resuscitation are pulmonary oedema, acute respiratory distress syndrome, abdominal compartment syndrome, peripheral compartment syndromes, elevated intraocular pressure, increase gut permeability and burn wound conversion (24, 35, 50). It also hampers burn wound healing contributing to worsening scar formation and potentially decreased physical function.

2.3.5.2 Resuscitation Fluid Choices

Fluid resuscitation is fundamental in the management of acute major burns. The two most common fluids administered during the resuscitation period are either crystalloid or lactate ringer solution (23). However there is ongoing debate regarding the use of and timely delivery of colloid (protein based) solutions.

Colloids are used to increase the intravascular osmolality and to stop the extravasation (leakage into the extracellular space) of the crystalloid or lactate ringer solution (23). There is conflicting evidence as to whether colloids decrease fluid volumes delivered in the initial resuscitation phase or add to already existing tissue

oedema. Goodwin et al (1983) and Jelenko et al (1978) supported the use of colloids suggesting they reduced fluid resuscitation volumes infused, however mortality and pulmonary complications were increased. There was also no significant reduction in systemic sepsis or need for escharotomy (15, 51, 52). Pham et al (2008) reviewed the literature and found several studies indicating colloids provide little clinical benefit to burn patients especially in the first 12 hours of resuscitation. Further its use has been shown to increase lung water content (pulmonary oedema) after the resuscitation phase even in the absence of an inhalation injury (51, 53).

Further experimental investigation is required to determine the most appropriate fluid resuscitation regime in order to limit tissue and lung oedema and the negative impact to the patient.

2.4 Impact Of Acute Oedema

There are numerous factors contributing to the magnitude of oedema in burn patients, both directly related to the extent or severity of the injury and the medical, nursing and allied health interventions or lack thereof (dressings, fluid resuscitation, oedema management procedures). Burn wound oedema can alter wound severity through increasing the oxygen diffusion distance to the wound, forming a physical barrier to healing (54). Consequently increasing the risk of hypertrophic scarring with associated functional, psychological and aesthetic sequelae (55). In children, a wound taking greater than ten days to heal had an eight percent chance of hypertrophic scarring (7). Finlay et al (2017) demonstrated reduced burn scar quality in adults as the time to healing increased, with the effect being significantly greater within 21 days after injury (56). Oedema affects the outcome of the wound (size, depth, healing). It also affects an individual's immediate physical function. Oedema can limit the range of motion of joints, cause pain with movement and mobilisation, increase the effort required to move and affect the cardio respiratory system if the lungs are involved. The changes in the composition of oedema in subacute or chronic states, may increase the resistance to movement (57). In addition, prolonged oedema has been associated with deposition of calcium in the tissues, fat in the muscles and peri muscular fascia thickening (58). To limit the negative impacts of oedema, time is of the essence and oedema management strategies should be instigated straight

away. Oedema assessment techniques are imperative to successful oedema reduction and prevention.

2.4.1 Summary

Many factors contribute to the degree and extent of oedema in this unique population and if it is not managed optimally the results can be devastating. Timely management of acute burn oedema can positively impact burn survivors' bio-psycho-social outcomes. Reducing wound healing time will also decrease the cost to the health system through decrease hospital inpatient length of stay, decreased services as an outpatient, decrease surgery cost and decreased risk of infections. Thus to improve acute and long term management of oedema an appropriate clinical tool for measuring and monitoring fluid shifts will help guide best practice.

2.5 Outcome Measures To Monitor Post Burn Oedema

The literature presents several options or current practices for quantifying oedema in both major and minor burns but they are not without limitations. The most common measures of burn fluid shift or oedema change are discussed below and it is evident a true gold standard outcome measure for burn oedema is still a goal worth pursuing.

2.5.1 Major Burns: Monitoring Of Resuscitation

Burns >15-20% TBSA require fluid replacement therapy to maintain circulating blood volumes. Initial volumes are determined by formulas including TBSA and patient weight variables. The fluid volume has to then be titrated according to the individual's response to therapy.

Endpoints of resuscitation include primarily urine output, and secondarily haemodynamic parameters such as blood pressure and oxygen saturation (44). Fluid is titrated based on maintaining a urine output of 30-50ml per hour (or 0.5-0.8 ml/kg/hr). The accuracy and validity of these endpoints of fluid resuscitation as a measure of whole body perfusion and fluid balance have been questioned (29). Burn centres are allowing urine output to exceed accepted values, contributing to over resuscitation (22, 41). Cartotto and Zhou (2010) carried out a retrospective review of

196 patients at a single centre over eight years and found the mean urine output was 1.2ml/kg/hr (SD 0.7) in the first 24 hours and 76% of patients received >4.3ml/kg/%TBSA of fluid (recommended 4ml/kg/%TBSA with Parkland formula) (41). Despite knowing the phenomenon of fluid creep, the burns centre did not adjust the resuscitation volumes to maintain urine output within the accepted range. Urine output has also been suggested to lag behind the actual events of hypoperfusion by up to two hours (21, 59).

Other options of endpoint fluid monitoring have been explored, but many of these are invasive and require expensive or specialist equipment (e.g. central venous catheters and pulmonary artery catheters) (45, 49, 60, 61). Burn patients requiring formal fluid resuscitation admitted to Fiona Stanley Hospital (FSH) may be treated on the burns unit and not the intensive care unit, so invasive monitoring such as Swan-Ganz (pulmonary artery) catheters are not available.

An American Burns Association survey of burn centres showed rates of different objective measures used to guide fluid volume titration are: pulmonary artery catheterisation eight percent, transpulmonary thermodilution three percent (44). These provide right heart diagnostic information to rapidly determine hemodynamic pressures, cardiac output, and mixed venous blood sampling; base deficit seven percent and lactate five percent (indicative of respiratory or metabolic compensations), lithium indicator dilution five percent (cardiac output measure), and haematocrit one percent. These are all used as indirect measures of the body's fluid volume, haemodynamic state or tissue perfusion.

These recommendations may need to be treated with caution in the first 24 hours as attempts to normalise the values can lead to over resuscitation and compartment syndromes (44). Abnormal arterial lactate and base excess values have been shown to correlate with the magnitude of injury and their failure to correct over time predicts mortality but there are no prospective studies to support their use to guide fluid resuscitation (53). The pathophysiology of burn shock creates a persistent hypovolemic state that gradually subsides, attempts at rapidly clearing anaerobic by products with aggressive volume replacement (attempting to normalise blood lactate and haematocrit) may be unsuccessful and exacerbate oedema formation.

Holm et al (2004) completed the only well designed prospective randomised trial comparing burn shock therapy guided by invasive haemodynamic monitoring to restore preload and cardiac output with standard therapy according to Parkland's formula (in the first 24 hours after burn) (60). In the pre-load driven intervention group intrathoracic blood volume or cardiac index (to within normal range) was unable to be achieved and they received 68% more fluid (above the predicted volumes) compared to the Parkland driven strategy. No association between increased fluid administration and more effective resuscitation was shown and the patients in the treatment group also showed much more pronounced subcutaneous oedema. This demonstrates attempts to normalise invasive haemodynamic properties may not lead to improved outcomes in the first 48 hours of major burns. It also has increase risks due do its invasive procedure and injection of contrast dye. Invasive procedures in acute burn care also increase the risk of septicaemia and wound infection (44).

Patient's weight can also be used to monitor changes in total body fluid although clinical validity is controversial (62). Reliable and valid body weight measurements are difficult to ascertain in the acute burns environment due to reduced patient mobility; resuscitation fluid retention and the added weight of burn dressings and wound ooze. All these factors introduce confounding and variability in the weight measurement and make interpretation of weight changes difficult.

Current techniques to guide fluid therapy are blunt and do not measure volumes of the body fluid compartments, most importantly extracellular and intracellular fluid. These give an indication of the extent of oedema (primarily in the ECF) and reabsorption of fluid into the capillaries and/or cellular oedema (23). If the volume of ECF could be measured easily and regularly over time, then re-hydration volumes could be adjusted to maintain normal (13-17L or ~25% of total body water) or clinically acceptable values, in turn limiting oedema and potentially catastrophic side effects (63).

2.5.2 Minor Burns: Localised Oedema Outcome Measures

Burn oedema volume and location (e.g. lungs, limbs) relates to the spectrum of severity of burn. In minor burns, complex, formal fluid resuscitation is not required

and oedema is generally localised to the vicinity of the burn site. The ability to track changes in localised oedema volumes at the site of injury can provide information on the efficacy of oedema management and treatment (e.g. medical management, physiotherapy input) and thus help guide best practice. An understanding of effective interventions is determined through appropriate assessment. There are few sensitive and accurate measures of localised oedema that are easy and quick to perform, however they are not without limitations in the burn trauma environment.

The 'gold standard' measures of peripheral oedema (limb volume change) include WVD and CLM (57). These can be difficult to perform with the nature of burn injury and can be logistically and mechanically challenging. For example, WVD may require large volumes of water to submerge a whole limb, depending on the location of burn and thus is not practical in large %TBSA burns. In addition, the vessel must be cleaned appropriately between subjects to prevent potential cross-infection. Limb circumference measures have limitations in the burn population due to dressings and open wounds. Infection prevention and management protocols of individual burn facilities, may prevent the use of CLM, otherwise require single use tape measures thus increasing patient contact consumables (64). In lymphoedema and hand therapy, however, CLM has been shown to have a significant correlation with WVD and thus can be used with confidence in detecting volume change (65, 66).

Limb oedema can also be objectively measured using clinical assessment, magnetic resonance imaging (MRI), computed topography scans, near-infrared spectroscopy (NIR), perometry and ultrasound. However, they lack clinical utility and validation (18, 67). An easy to use, rapid outcome measure for more localised oedema will provide immediate feedback of the effectiveness of oedema and wound management interventions.

2.5.3 Wound Healing Assessment

Wound healing is a significant component in recovery from burn injury and it is also influenced by oedema change. Time to wound healing is directly related to the severity of scarring (56). Monitoring of healing is essential to ensure the most appropriate intervention to promote healing is carried out. This includes the choice of

dressing, surgical intervention, use of pharmaceutical agents (e.g. antibiotics), other indicated medical management (e.g. vascular optimisation), and oedema control.

Current monitoring of wound healing assesses wound size, wound bed characteristics, type of tissue, colour and wound bed depth. Various methods including clinical assessment, photographs, visitrak wound area tracing, circumference measures, computer software packages such as digital planimetry and image J may be used in isolation or combination (68, 69). None of these methods provide cellular level information of the wound, have low sensitivity to changes or are expensive and require an undressed wound.

Laser Doppler perfusion imaging (LDPI) is another method utilised to determine burn wound depth. It operates by scanning a burned area with laser light and the light frequency changes with the amount of perfusion of the tissues. A color-coded perfusion map is generated, which corresponds to varying burn depths. It is a highly valid and accurate (> 95%) measure of burn wound depth (70, 71). Wound infection, tissue curvature, topical substances and ambient light significantly affect the accuracy of LDPI, major limitations to its use in burns. (20). Other optical techniques such as optical coherence tomography, reflection-optical multispectral imaging and orthogonal polarization spectral imaging are non invasive, rapid methods of burn wound depth assessment (20). Their application and use are still in the research phase. Thermographic imagery is another emergent burn wound evaluation tool, however it is limited to use in temperature controlled rooms with a constant humidity (72).

The literature transcribes, ‘no method of measurement is perfect’ and results need to be interpreted in conjunction with the clinical picture (69). An instrument that provides insights into the cell health and processes of healing over time would facilitate successful clinical decision making.

2.5.4 Summary

There is a lack of user friendly, valid, reliable and non-invasive outcome measures to determine real time fluid changes after an injury and during wound healing in an

acute setting. Bioimpedance spectroscopy (BIS) may be a solution. It is a tool capable of oedema assessment and emerging as an indicator of wound healing.

2.6 Bioimpedance Spectroscopy

There have been few studies investigating bioimpedance and measurement of inter-compartmental fluid levels in major burns, and the current outcome measures of fluid management in this challenging population in the acute and subacute phase have questionable accuracy. Due to the rapid acute change and shift of fluid into tissues secondary to the body's response to injury, the potential ability of BIS to provide 'real time' measurements of volume change is promising. The first studies exploring bioimpedance as a method of oedema assessment in major burns was in the late nineties (73, 74). Bioimpedance analysis was determined to be a reliable and sensitive measure of TBF volumes. However, further investigation of BIA, as a method of monitoring fluid resuscitation, did not occur until ten years later (75). Was this potentially due to the difficulties in clinical application of BIA in the burns environment? The current literature explores the utility of BIS in a range of clinical areas, with novel concepts that may be applied in the burns patients. Following on, BIS and its potential application in the burns environment is described.

2.6.1 Use And Significance Of Bioimpedance Spectroscopy In Burns

Bioimpedance Spectroscopy is a method used commonly to assess body composition and allows for evaluation of specific body compartments and cell health such as fat free mass (FFM), inter-compartmental fluid volumes (ECF, ICF, TBF) and cell mass (76, 77). It is used commonly in the areas of nutrition and physical health and has gained popularity as a clinical tool in the last two decades. It is routinely used to monitor and assess lymphoedema (78, 79) and has also been used extensively in studies investigating fluid shifts in haemodialysis, as a prognostic tool in human immune-deficiency virus (HIV) and cancer, and as a screening tool for malnutrition in the elderly (80-82). The method has been validated in healthy and clinical populations against MRI and bromide and potassium dilution techniques, which are considered gold standard in the assessment of fluid compartment volumes and lean

body mass (LBM) (83-86). Bioimpedance spectroscopy is a popular tool for monitoring and assessing clinical changes as it is easy to use, processes information rapidly at the bedside, is relatively inexpensive and is portable (Figure 2.5) (87). It has demonstrated sensitivity, repeatability and high reliability of measures all deemed essential when investigating a new method of measurement (79).

Figure 2.5: Impedimed SFB7 bioimpedance spectroscopy instrument (Impedimed Limited, Brisbane, Australia)

Bioimpedance has demonstrated its application and usefulness as an assessment tool in fluid monitoring, wound healing and nutritional assessment in various clinical settings (88-91). Investigation into the possible uses of BIS in burns is therefore warranted. The burn wound journey can be arduous and long. The injury itself causes, whole body and local fluid shift alterations, increased metabolic rate and protein catabolism (affecting LBM) and open wounds (92). There is no real time, clinically available tool in the burns environment that objectively measure changes in wound healing, fluid distribution and LBM. The following provides an overview of BIS and discusses the possible applications and limitations of BIS in the burns populations.

The term bioimpedance describes the response of a living organism to an externally applied alternating electrical current and is a measure of the opposition to the flow of that electrical current through the various tissues (93). It works on the principle that human tissues have different resistive and conductive properties. Electrically, a cell

can be represented as an "ion-rich conductive centre (cytoplasm) embedded in an ion-rich conductive medium (extracellular fluid), separated by a relatively non-conductive barrier (cell membrane)"(94). Because the conductivity of the body is directly proportional to the amount of electrolyte-rich fluid that is present, BIS can be used to measure fluid components such as TBF and the condition of the tissue (85).

There are a number of bioimpedance instruments on the market and they differ in the type of and array of electrodes used, range of frequencies applied and mathematical formulas (regression derived or biophysical curve fitted modelling) that are used to determine the body composition values (95, 96). They are single frequency, multiple-frequency and spectroscopy devices. Irrespective of the device, bioimpedance works by applying electrodes to intact skin and then a small, painless alternating current across one or more frequencies is passed through the body. The current flows depending on the composition of the body. The resistance (opposition of flow to an alternating current) and capacitance (delay in the passage of current through the cell membranes and tissue interfaces) of the tissues and bodily fluids also vary with the frequency of the applied electrical current (76). This necessitates an understanding Ohm's law which states that the flow of an electrical current (I) passing through two points of a conductor is equal to the voltage drop (V) divided by the electrical resistance (R) between these 2 points (97).

I = V/R **Or** R = V/I

This is based on a direct current into a simple conductor. Generalisation of Ohm's law to alternating current yields the concept of electrical impedance (Z).

$$Z = \frac{V}{I}$$

At low frequencies (<30-50 kHz) the current passes through only the ionic environment surrounding or outside the cells and therefore is indicative of ECF. At high frequencies the current passes through the ionic extracellular environment, the cell membranes and intracellular environment and is indicative of TBF. Intracellular fluid can be determined by subtracting ECF from TBF and is also reflective of body

cell mass (BCM) (89). The components of the individual fluid compartments are as follows (Figure 2.6). *ECF* (13-17L): Fluid outside the cell consists of interstitial fluid (~13L) (dense connective tissue and bone), plasma (~3L) and transcellular fluid (~1L). It has a high electrolyte content of which 90% is Na^+, then Cl^- and HCO_3 and traces of others. It will expand in conditions of hyper-hydration and decrease in hypo-hydration states; *ICF* (21-25L): Fluid contained within the cell, has a high K^+ content (95%) as well as Mg^+, phosphates and protein; *TBF* = ECF + ICF. 56%-70% of the body consist of fluid, equivalent to 35-45L (97, 98).

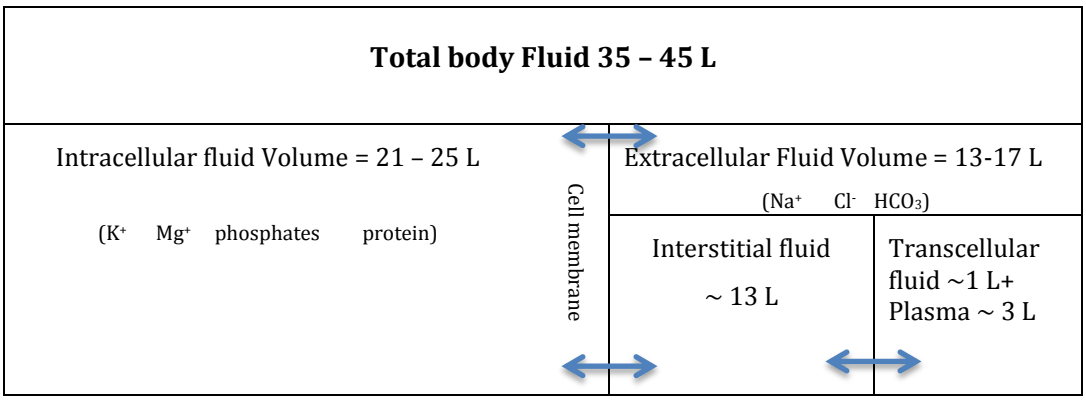

Figure 2.6: Body fluid compartments

The raw bioimpedance variables of resistance (R), reactance (Xc) and phase angle (PA) provide information about tissue hydration and integrity. Resistance is reflective of the body's water compartments and is inversely proportional to fluid volume and therefore oedema i.e. the greater the fluid the lower the R (85, 89). Reactance indicates cell mass and function. Phase angle is the arc tangent of the ratio Xc/R (99) and is a result of the capacitance (a factor in determining Xc), due to the structure of the cell membrane. Capacitance causes the current to lag behind the voltage creating a phase shift (100, 101). If tissue health (integrity of the cytoplasm, cell membrane and/or cellular fluid) is disturbed in any way (e.g. inflammation, disease) the electrical properties of those tissues are altered, therefore directly affecting PA (100). Tissue or cell damage results in a loss of cell membrane structure, which allows ions and the BIS current to pass through the cell. Damaged cells therefore behave more like a resistor than a capacitor. Phase angle therefore relates to the health of the cell/s (a lower PA is indicative of poorer health) (89).

These raw variables can either be interpreted alone or are used to calculate the fluid volumes through empirical predictive equations (76, 102).

The different bioimpedance devices use different prediction equations but the impedimed SFB7 BIS instrument, uses a model which has been reported as being the superior model under conditions in which body water compartmentalisation is altered from normal state (103, 104). It also does not require the use of population specific prediction equations like single frequency bioimpedance analysis (BIA) does (105). The raw impedimed SFB7 BIS resistance values indicative of ECF, ICF and TBF are obtained on the basis of the Cole-Cole model and from here on BIS refers to measures obtained from this model (106). For further insight into the different models refer to Kyle et al (2004) and Mulasai et al (2014) (76, 107). Bioimpedance spectroscopy and multi-frequency BIA feed seven or more (4-1000kHz) and two or more frequencies (4-100khz) respectively into the tissue allowing for measurement of ECF, ICF and TBF (108). At low frequencies the current can penetrate the ECF only, due to the high capacitance of the cell membrane and at high frequencies it passes through both the ECF and ICF measuring TBF. The ICF is then determined by subtracting ECF from TBF. Single frequency BIA feeds one current, most commonly 50 kHz, which penetrates both the ECF and ICF (102). It is therefore more suitable for TBF and fat free mass (FFM) estimates only (107, 109).

Body composition analysis can be performed via whole body or segmental BIS, differing in the placement of electrodes. Whole body BIS involves four electrodes (two current sensing and two current drives), which are placed on intact skin in a standard tetrapolar configuration on the dorsal surface of the hand and feet (104). It is standardised and has been widely used in assessment of physiological changes at a whole body level in normal and specific clinical populations (Figure 2.7) (83, 110).

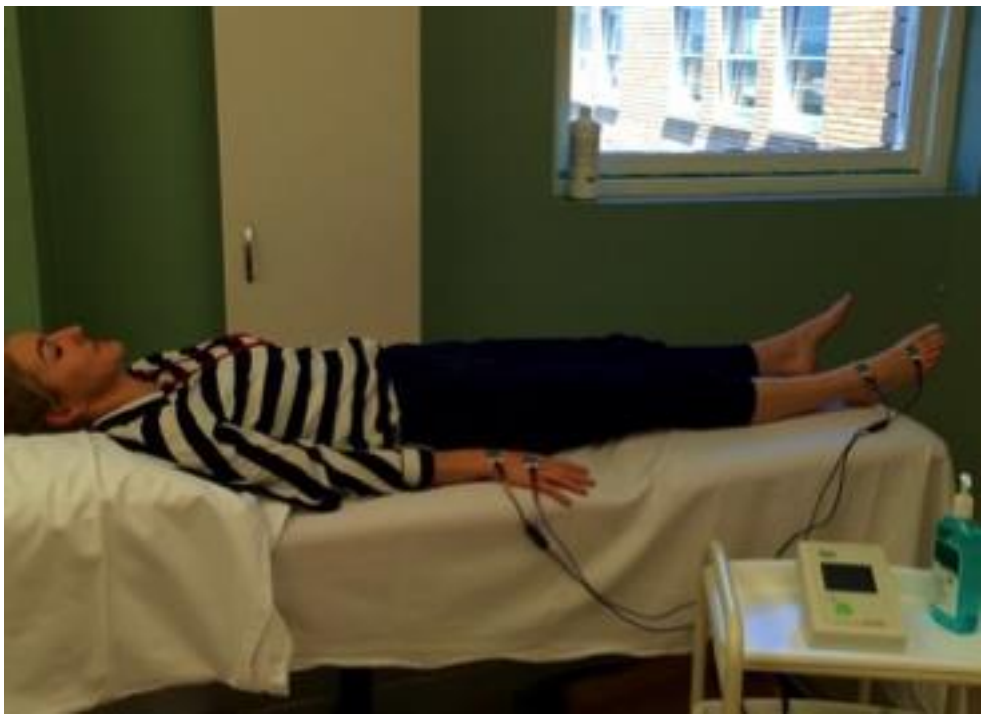

Figure 2.7: Bioimpedance spectroscopy: whole body electrode positions

Segmental BIA measures arm, leg or trunk segments and requires drive electrodes to be placed on the dorsum of the ipsilateral hand and foot (as per whole body BIS) and sense electrodes placed on either the 1) the dorsum of the feet at the talo-crural joint or 2) the dorsum of the hands at the radio-ulnar joint, depending on which segment is being measured (76). To produce interpretable data, this method relies on theory of equi-potentials, which are loci of points with the same potential and are perpendicular to the flow of current (111).For further explanation please refer to Cornish et al (1999) (112). Segmental BIS is described as being insensitive to oedema of the contra-lateral limb (113). It has been used in the assessment of unilateral lymphoedema to generate ECW/ICW ratios, using the unaffected arm as a control (114).

Localised BIS is a relatively new concept and involves the electrodes being placed close to the site of injury (e.g. pressure sore, muscle tear, fracture site), zoning in on the field of assessment (87, 115, 116). Electrode positions have not been standardised, i.e. sense electrodes are placed as close to the injury as possible with the drive electrode remaining in the standard positions (hand and feet), or moved alongside the sense electrodes, or either side of a sub-limb segment e.g. calf (Figure 2.8) (117). When the distance between the sense electrodes is reduced the sensitivity

close to the electrodes increases but the measurement depth is reduced (118). In muscle injuries, single frequency BIA with localised novel electrode placement (sense and drive either side of the injury) was able to detect changes in oedema and cellular injury consistent with MRI imaging over time in the individual (115). Other BIS studies assessing single limb oedema use segmental or localised electrode placement as whole body electrode placement has been shown to be insensitive to decreases in volumetric measures such as during the treatment of lymphedema (113, 119). The reason for this is suggested to be due to the electric current path through soft tissues being largely determined by body geometry and the relative contribution of body segments to the whole body BIS measures.

Impedance measurements of a sublimb (localised) segment, the calf, have become the method of choice to monitor fluid status during dialysis and are more sensitive and precise than whole body or limb measurements (95, 117).

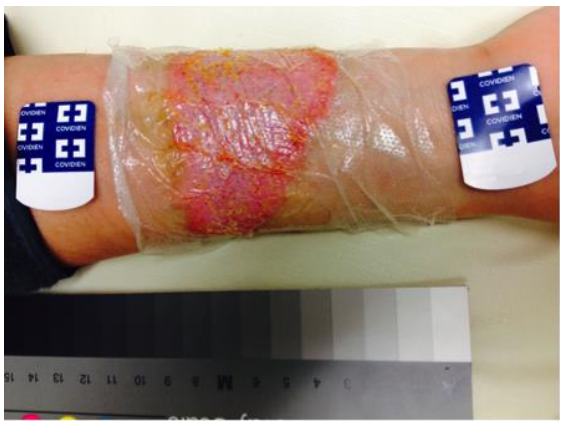

Figure 2.8: Localised (sense) electrode placement either side of a wound

In the burn environment, the choice of electrode placements would depend on the desired assessment, such as whole body fluid shifts or local wound oedema. The standardised electrode positions may pose a barrier to clinical use in burn injured patients. This will be discussed in more detail later.

2.6.2 Application Of Bioimpedance Spectroscopy To Clinical Practice In Burns

2.6.2.1 Monitoring And Assessing Fluid Shifts

In a major burn, fluid resuscitation is instigated to maintain circulating blood volume, prevent hypovolemia and ensure adequate tissue perfusion and blood supply to vital organs (22, 40, 120). Fluid volumes resuscitated need to be monitored closely and administered as close to predicted as possible to prevent burn shock, renal failure, compartment syndromes, burn wound conversion, respiratory compromise and even death (29). Too much fluid will add to the already significant oedema volume.

Currently fluid resuscitation volumes in major acute burns are initially determined using formulas as guidelines and then titrated primarily according to hourly urine output (38). Urine output is a quasi- measure of fluid shift and is not in real time. Other observations utilised as indirect measures of fluid management and titration are blood serum levels, standard nursing observations (blood pressure, tissue oxygenation) and measurement of cardiac output with pulmonary artery catheters (44, 45, 49, 60). Some of these are invasive and attempts to normalise bloods and cardiac output in first 24-48hrs often leads to increased fluid volumes delivered, thus increased oedema, but not improved patient outcomes (60). Where invasive monitoring is not available, the clinician's ability to respond quickly is compromised by the insensitive measures available.

Only a few studies have utilised BIA in the assessment of fluid shifts in burns patients (73-75). It has however, been investigated in goal directed therapy to guide intraoperative fluid administration in surgical and intensive care unit patients with promising results (89, 121). Both Ernstbrunner et al (2014) and Malbrain et al (2014) believe BIS can help guide fluid resuscitation but suggest more research is needed in the critically ill population (89, 122). Ernstbrunner et al (2014) assessed volume status in patient's before and after surgery using BIS and believe it could become a useful guide to intraoperative fluid therapy (122). They found ECF ($p<0.05$) increased significantly from before to after surgery with administration of intraoperative fluid (mean 1.9L) with no significant change in ICF ($p=0.15$). In

contrast, Plank et al (1998) found BIS underestimated absolute volumes of ECF when compared to dilution techniques in the critically ill from day 0 to 10. However, the ECF change from day 0 to 10 was not significantly different between BIS and the dilution method (123).

A study by Slotwenski et al (2013) of critically ill patients found those with sepsis had significantly higher impedance (566 ± 98.66 ohms, P=0.0003) than those with severe sepsis (423.86 ± 149.7 ohms), and a lower % ECF (45.95± 2.97% vs. 49.2 ± 6.11% P=0.026) (124). Summarising, those with increasing sepsis severity had decreasing impedance and a greater percentage of ECF. This may be explained by damage of the cell membrane and loss of cell wall integrity in the critically ill (89).

Assessment and monitoring of dry weight (targeted optimal body weight of the patient, achieved through the removal of excess water) in dialysis is important and BIS can provide real time continuous measurements of compartmental fluid volume changes and calculate over-hydration within 1-2 L (considered a clinically appropriate range) (125). Others have found development of their own bioimpedance algorithms improves accuracy of fluid volume changes in dialysis patients (90). Raimann et al (2013) recently compared single frequency BIA and BIS to direct estimation methods (DEMs) (i.e. deuteriumoxide-dilution, bromide-dilution and total potassium) in haemodialysis patients (126). They found BIS ECF was closer to DEM ECF than single frequency BIA based on root mean squared error analysis. Both BIS and single frequency BIA were equally precise in determining ICF and TBF, when compared to DEMs.

Close monitoring of net fluid shifts in large burns is essential, especially in the first 24-48 hours, when a complex inflammatory process is in place and fluid shifts are great with ebbs and flows. Using BIS to ensure adequate intravascular blood volume may be achieved through titrating fluid volumes to achieve a stable and ‘normal range’ ICF volume. Once the target ICF is reached and maintained the resuscitation fluid volumes may be titrated with the aim of reducing ECF volumes. This is one potential way BIS may be utilised for, real time monitoring to optimise fluid resuscitation and therefore improve patient outcomes.

2.6.2.2 Monitoring Peripheral Oedema

As well as fluid shifts in large burns, monitoring smaller fluid shifts, or oedema, in minor burns is just as important. The standard measures of peripheral oedema include clinical assessment, water displacement volumetry (WDV) and circumference limb measures (CLM). These can be difficult to perform with the nature of burn injury and can be logistically and mechanically challenging. In a proof of concept study, Edgar et al (2013) demonstrated BIS is a sensitive measure of small changes in fluid locally and thus ideal for monitoring and determining best practice for oedema management (75). Bioimpedance spectroscopy is used routinely to monitor and assess lymphoedema (78, 127, 128). Multiple frequency BIA was 100% sensitive in detecting limb volume changes as compared to CLM in upper limb lymphoedema (81) and it is now being considered as gold standard of measurement in lymphoedema (97).

Segmental BIS was able to detect changes in oedema post-traumatic ankle fracture with a strong inverse linear relationship between impedance at 5kHz (representative of ECF) and WDV (r=-0.92) (67). Localised electrode placement BIS was also able to detect changes in oedema and cellular injury consistent with MRI imaging over time after muscle injury (115). Pichonnaz et al (2015) propose the raw BIS variable R_0 had greater diagnostic sensitivity and responsive, and is a valid method for measuring oedema post total knee replacement, as compared to CLM and volume measures (129).

An accurate and sensitive assessment technique of oedema can guide best practice in the treatment of oedema, thus contributing to optimum healing conditions in most injuries. Bioimpedance spectroscopy has been identified as a method of oedema assessment that has merit after burns (75).

2.6.2.3 Wound Assessment And Monitoring

Optimal management of the acute burn wound aims to: cool the wound immediately for 20 minutes; reduce oedema in the first three to four days; and prevent burn wound conversion in order to aid in reduced healing times (4, 17). This is important because the severity of scarring is directly related to time to healing (25). Current

wound assessment techniques can be time consuming, require specialist equipment, software or clinicians and many do not provide outcomes that are indicative of the wound at a cellular level. The most common techniques are photographs, laser Doppler and wound area measures, all reliant on a degree of clinician experience and subjectivity. Objective assessment of wound healing is essential to evaluate nutritional and therapeutic interventions and detect complications. Bioimpedance analysis is an emergent concept in the assessment of wound healing but shows promising results.

Lukaski et al (2012) discovered in several case studies in wounds of varying aetiologies, localised single frequency raw bioimpedance variables, resistance (R), reactance (Xc) and phase angle (PA) increased with re- epithelialisation of the wound and could detect the presence of infection prior to laboratory methods (130). This is supported by Moore et al (2011) who found PA measurements mirrored the health of the wound and provided an accurate tool for assessing the regional tissue health, in diabetic, surgical, neurotrophic, venous, traumatic and infectious chronic wounds (88).

Wagner et al (1996) found localised BIA (frequency 50kHz) was able to predict patients at risk of pressure ulcers (116). Phase sensitive measures were taken and patients at risk had significantly decreased Xc, R and PA values suggesting malnutrition, ECF accumulation and decreased cellular vitality. In rats, local BIA was found to be a highly reliable measure with low within subject variability and high retest reliability for describing cellular changes that occur during and signal complications to wound healing. In these rats, tissue health was highly correlated with impedance (94). Rats were subject to a one and three hour ischaemic injury with weighted magnets and fluorescence angiography was utilized to image real-time blood flow in the tissue. Wounded areas showed a decrease in impedance magnitude and PA closer to zero, suggesting BIS could identify tissue damage that is not visible. Swisher et al (2015) reported that many researchers are actively exploring this area, with a number of clinical trials underway and impedance-based wound monitoring devices have been patented (94).

Bioimpedance assessment decreases the degree of subjective error in wound assessment and may allow for earlier detection of infections and more timely

treatment as opposed to waiting for clinical signs and laboratory tests in the burn population. The ability to track wound healing with an instrument, which is indicative of wound changes at a cellular level could positively affect treatment choices. Major burns patients are a high risk for pressure injuries, especially in the intensive care setting. Regular assessment of at risk areas such as the heels and the sacrum with BIS could lead to earlier pressure care intervention, such as more frequent patient turns, thus minimising the impact on patient care and morbidity. In the series of the following studies, one explores the novel concept of wound assessment in minor limb burns.

2.6.2.4 Assessment Of Nourishment And Health

It is known that a burn injury causes an increase in metabolic rate and catabolism of protein, hence the need for increased nutritional and energy requirements (8). This response is characterised by decreases in lean body and total body mass, liver dysfunction, proteolysis and insulin resistance amongst other things (131). Newsome et al (1973) stated severely burned patients in the acute setting can lose up to 25% total body mass (132), as skeletal muscle is a major source of fuel for the burned patient (133). Accurate assessment of cellular level body components such as body cell mass (BCM which is equivalent to ICF) and fluid compartment volumes can indicate malnourishment and cell health. Malnutrition has been associated with increased infections, longer length of hospital stay and higher mortality (134-136). Therefore optimising nutrition is essential to promote and provide best conditions for wound healing, help prevent infection and limit functional decline (137). Measuring BCM can provide an estimate of protein balance and an aspect of metabolic improvement (138). Protein is the main component of muscle mass and protein is directly related to ICF. Therefore, an improvement in BCM or ICF may indicate the effectiveness of nutritional support (139, 140).

Initial nutritional support assessment of burns patients includes consideration of resting energy expenditure, burn depth, %TBSA, time post burn, pre-existing nutrition and their level of activity. These factors are then incorporated into equations such as the Toronto formula and modified Harris-Benedict equation to determine the caloric requirements (141). These assessments are used in the BSWA. Ongoing monitoring and assessment are required to adjust nutritional support as

necessary. Other methods of nutrition and LBM assessment may include: questionnaires (e.g. Subjective global assessment) however these can be time consuming and tedious for the patient to complete and clinician to assess, biochemical indicators such as serum albumin, total lymphocyte count and serum pre-albumin but there have been recent objections raised against these as they are influenced by acute inflammation (142, 143). Computerised tomography (CT) and MRI can also assess LBM but these are costly, time consuming, not always readily available and may not be appropriate for acute burns patients, particularly the critically ill. Ongoing monitoring of nutritional status is by body weight, aiming for a stable positive change. Factors such as maintenance fluids, fluid shifts associated with infection and hypoproteinemia however can mask LBM losses as fluid can increase their weight (131).

Bioimpedance spectroscopy has the ability to assess components of nutrition with variables BCM and PA. Phase angle indicates cell viability and health. Bioimpedance analysis, using a predictive regression equation, has been shown to provide valid estimates of skeletal muscle mass (SMM) (r^2 = 0.86, SEE 9%), across multiple ethnicities, when compared to SMM determined by MRI (85). In disease such as HIV and cancer, a loss of ICF (reflecting BCM) is frequently accompanied by an increase in ECF. This can cause body weight to remain constant or increase, masking malnutrition (144), indicating BIS may be useful in the assessment and monitoring of burn patient caloric intake or nutrition.

In studies particularly in the elderly a lower PA is associated with malnourishment and has been found to be a determinant of those nutritionally at risk in hospital (137). Zdolsek et al. (1998) proposed that PA was able to detect the effects of a burn and sepsis in cellular membranes, because it significantly decreased in the post-burn period, with the lowest values being found in two patients who died (74). This was however a small sample (n=10). In critically ill patients in intensive care units, Lee et al (2015) found a PA <4.1 (+-1.1, P=0.01) degrees indicates negative nutritional issues (145). In another study, lung cancer patients with a PA <4.5 degrees had significantly (P=0.01) shorter survival rates (median 3.7 months) compared to those with PA >4.5 degrees (median 12.1 months) (146). The PA of healthy white populations has been reported as >7 degrees for males and >6 degrees for females

(101, 147). Others state the average range of PA for healthy humans is 5-9 degrees (148). Multiple frequency BIA was used to assess Xc and PA in response to re-feeding treatments for anorexia nervosa patients (n=21) (149). Reactance and PA improved significantly in patients who were receiving the treatment and these values no longer differed from age matched healthy females at 15 weeks, even though their body mass index remained significantly lower than the controls. This suggests cell health can improve without an increase body weight. However, this was a small sample size.

In burns it is essential patients have optimal feeding to promote and provide best conditions for wound healing, help prevent infection and limit functional decline (137). It is difficult to determine an individual's nutritional needs and absorption of their dietary intake, especially in major burns, due to other medical issues. Assessment of BIS variables could theoretically guide nutritional support prescription and aid in optimising their management (150). Investigation of nutritional assessment in burns is out of the scope of this study but is an area worth pursuing in future research.

2.6.2.5 Assessment And Monitoring Of Body Composition

Part of the standard care of patients with a burn injury is exercise to maintain movement, function and strength. It is essential to patient recovery, for optimal outcomes, in both the acute and long term rehabilitation phase (151). There is limited literature in acute burns, assessing the impact of exercise on the individual's rate of protein catabolism and lean body mass (LBM), especially in the acute care phase.

A burn injury causes an increase in metabolic rate and catabolism of protein. This response may last up to or greater than 12 months after injury (152, 153). The loss of protein leads to a loss of LBM and muscle wasting and therefore strength. The lean tissue compartment (BCM) is vitally important in the body's ability to respond to acute and chronic illness. A decline in BCM is associated with a decrease in strength, functional decline, and immune function, as seen in HIV patients for example (144, 154, 155). The addition of exercise will enhance an individual's energy requirements but is essential in building or maintaining lean muscle mass. It is however unknown whether a resistance and strength building exercise program in the acute burn phase

is beneficial or detrimental to the healing process. Also for a patient to be able to participate fully in physical rehabilitation they need enough caloric energy to do so. Bioimpedance spectroscopy provides an opportunity to objectively measure the effects of exercise on LBM and more specifically BCM.

Current and traditional methods of monitoring the effects of exercise training on muscle and LBM are, anthropometric measures (girths and skin folds), muscle strength (by e.g. dynamometry), dual X-ray absorptiometry, MRI and CT. Only MRI and CT can provide muscle anatomical and physiological cross sectional area but these aren't always readily available for use and are expensive (91). Dual X-ray absorptiometry is not real time and involves the injection of tracer dyes; skin folds are challenging, if not invalid, with open wounds or scarred tissue; and muscle strength dynamometry is a valid measure, but may at times be limited due to pain with open wounds in the acute burn environment.

Bioimpedance spectroscopy has been used to assess both body composition and the effects of exercise training on body composition in healthy and clinical populations in numerous studies (110, 156, 157). Weber-Lang (2009) showed improved BCM over time when comparing two different exercise training types in end stage lung disease (158). Intracellular fluid and SMM, assessed by BIA, increased significantly ($P = <0.05$) in both men (8.2% and 4.2% respectively) and women (11% and 3.9% respectively) after 16 weeks of resistance training (159). A significant correlation (Pearson $r= 0.66\text{-}0.8$, 95% CI, $p<0.01$) was found between upper extremity strength and SMM measured by segmental BIA in healthy individuals (91). Burn injured patients more than 2 years after injury, after a 12 week interval training program, displayed the same training effects, in strength measured by dynamometry and LBM measured by DEXA, as healthy matched controls (160). Immediate assessment of LBM by BIA would be far more convenient and would have less impact on patient's time than methods such as dual X-ray absorptiometry and MRI's.

Bioimpedance spectroscopy may provide an additional and complementary outcome measure for measuring the training effect of muscle. Some traditional anthropometric measures such as skin fold and girth measurements may not be suitable in large burns due to open wounds, extensive scarring, oedema and the loss of skin elasticity. Monitoring training effects of LBM and BCM with bioimpedance can be frequent

and easily achieved. It may therefore help guide best practice for exercise training. Additionally it may help determine how exercise affects BCM and therefore its potential impacts on nutrition and wound healing. The scope of this study however, does not include exploration of BIS in monitoring exercise training. It is important to understand though, the possible applications of BIS in the burns environment and how valuable it may be.

2.6.3 Limitations Of The BIS Technique And Its Use In Burns

The bioimpedance technique recommends the use of standardised electrode placement, correct positioning and preparation of the patient. Acute burn injuries will often preclude the use of standardised whole body and segmental electrode placements, due to a high percentage of injuries, hence open wounds, to certain areas on the hands and feet. Alternate placements need to be considered, deciphered and interpreted in this population. Stahn et al (2008) and Grisbrook et al (2015) report alternate electrode positions used on the upper limb are valid substitute however further research is needed for valid alternate positions on the lower limb (160, 161). The effect of mature scar tissue and the skin area of non standard electrode positions on tissue impedance and skin resistivity is also unknown. It has been shown that impedance is affected by the thickness of the stratum corneum of glabrous skin (162), which likely indicates there will be impedance differences in scar tissue.

Acute burns require dressings to assist wound healing and protect against infection. Common dressings used are nanocrystalline silver impregnated (a conductive material) and hydrocolloid (water based) dressings. Both of these dressings have the potential to alter the BIS variable measures as the technology is based on the conduction of a small alternating electrical current delivered through the body and is directly related to the ionic fluid in the field of measure. Grisbrook et al (2016) found Acticoat^{TM}, a silver dressing, significantly affected BIS fluid volumes (ECF, ICF and TBF, $p<0.01$) compared to no dressing in burns patients with a median total body surface area (TBSA) of 15% (163). However they did not include fluid resuscitation in their analysis. To account for the Acticoat^{TM} they developed an algorithm to adjust BIS variables for clinical interpretation. As previously mentioned, Edgar et al (2009)

determined whole body BIS was more sensitive and reliable in new dressings (<8 hour after application) compared to old dressings (>8 hours after application) (75).

Further understanding of the effect of various dressings on BIS variable outputs, across the spectrum of burns severity and phases of healing is required to enhance its clinical utility in this environment.

2.7 Summary

There is currently no single use, rapid measure of fluid volume change in the burns environment that can be utilised with dressings in place. Bioimpedance spectroscopy is a promising and novel measure of fluid shifts, wound healing, nutrition and training effects in burns and is worth the further investigation given in this particular research. The current research explores whether BIS is a reliable and valid tool in the assessment of fluid change and wound healing across the spectrum of acute burns, and addresses potential barriers to the use of BIS in this population. Bedside, user-friendly outcome measures of oedema will aid in management and limitation of the negative sequelae of burn injuries.

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Chapter 3 Addressing The Barriers To Bioimpedance Spectroscopy Use In Major Burns: Alternate Electrode Placement

This manuscript (Study 1) was accepted for publication in the Journal of Burn Care and Research, in February 2017.

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The PhD candidate, Pippa Kenworthy led the study and drafting of the manuscript and completed the submission, accounting for ~85% of the intellectual property associated with the final manuscript. Collectively the remaining authors contributed 15%.

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Foreword

The first two studies in this thesis investigate the use of BIS in acute major burns. They address the potential barriers to its use and the reliability and validity of BIS as a measure of fluid shift. The following study firstly investigates potential barriers to the use of BIS in acute major burns. Major acute burns, admitted to the BSWA, have Acticoat^{TM} (silver dressings) insitu and often have wounds at the site of BIS standardised electrode placement rendering BIS unusable in this environment. Alternative electrode positions have been investigated in healthy populations, but not in the burns environment. Therefore, prior to determining BIS reliability and validity in major burns and to enhance its clinical utility we investigated whether BIS alternate electrode positions were a comparable alternative to standardised positions, across different dressing conditions, in acute major burns.

3.1 Introduction

Bioimpedance spectroscopy (BIS) is a method of body composition analysis, which allows the immediate assessment of the inter-compartmental fluid volumes such as ECF, intracellular fluid (ICF) and total body fluid (TBF) and measures of cell health and function (1). By applying a small alternating current via electrodes placed on intact skin across a number of different frequencies, BIS measures the body's 1) resistance (opposition by a conductor) and 2) reactance (opposition by a capacitor) to the flow of an electrical current (2, 3). The frequency of the alternating current determines whether it can penetrate the cell membrane and at low frequencies it cannot (1). The BIS instrument uses a Cole-Cole (4) model to generate Cole model terms including, resistance at zero frequency (R_0) and at infinite frequency (R_{inf}), which are representative of ECF and TBF respectively and R_i (associated with intracellular fluid) (5). Fluid volumes (litres) are determined by applying the Cole model terms to predictive mixture theory equations incorporated into the BIS instrument (6).

The ability of BIS to measure real time fluid shifts non-invasively has led to numerous studies investigating its evaluation in different clinical conditions with a small number being conducted in the burns environment (7-10). In minor burns BIS is reliable and able to measure the direction of oedema change using localised electrode placement in any dressing condition (11). Grisbrook et al (2016) also showed whole body BIS can measure resistance and fluid parameters in burns with a median TBSA of 15% in the presence of Acticoat™ (Smith & Nephew, Australia) dressings, if the BIS variables are adjusted for using their provided Acticoat™ BIS algorithm (12).

There are numerous challenges in the assessment of fluid shifts in patients with burns, including (but not limited to) open wounds, dressings, reduced mobility plus 'the need to monitor small whole body fluid shifts on the background of large fluid resuscitative volumes(10). On average, 23% of burn injuries in the State Burns Service of Western Australia have either their hands or feet involved, thus preventing the standardised positioning of electrodes (13). Tetrapolar electrode placement for whole body and limb segmental BIS measures requires one current and one sense electrode 5cm apart to be placed on the dorsum of both the hands and feet on intact skin (14). Cornish et al

1999 suggests, based on the theory of equi-potentials (loci of points with the same potential and are perpendicular to the flow of current), movement of electrodes anterior, posterior or laterally will yield the same results as standard electrode placements (15). Whether this is practically valid is yet unknown. However, others have reported movement of electrodes proximally by 1cm and 2 cm can result in a change of mean resistance values by 2% and 4% respectively (16).

Dressings also need to be considered when using BIS. Moderate to large burns patients have an Acticoat^{TM} dressing insitu in the first 48 hours of burn care, the standard dressing used in the Burns Service of Western Australia. The dressings are in place at all times except when they are having a dressing change and shower. Thus, it is necessary to understand if standard and alternate electrode positions are comparable with dressings insitu.

The aims of this study were therefore to determine whether alternate electrode configurations for whole body and limb segmental BIS outputs are comparable to standardised electrode configurations in moderate to large size burns across different dressing conditions.

3.2 Methods

3.2.1 Participants

A longitudinal, prospective, single service study was conducted between December 2014 and February 2016. Patients admitted with an acute burn requiring formal fluid resuscitation were recruited to the study within 48 hours of injury, providing they were over eighteen years old and were able to provide written consent. They were excluded if they had hand and/or feet burns preventing placement of electrodes. Manufacturer's contraindications also excluded pregnant or breast-feeding patients, patients with surgical implants, cardiac pacemakers and/or on electronic life support devices.

Patients were initially recruited from the inpatient Burns Unit at Royal Perth Hospital (RPH) and then at Fiona Stanley Hospital (FSH) after the move of the Western

Australian state Burn Service to the new facility. There was no change to the study protocol, patient population or equipment used in the study.

3.2.2 Equipment

The ImpediMed SFB7 (ImpediMed, Brisbane, Queensland, Australia) was used to collect whole body and segmental BIS measures.

The equipment applies 256 discrete current frequencies (4-1000 Hz) to interpret each measurement. BIS computes raw variables (resistance, reactance) and derived fluid distribution values such as whole body ECF, ICF, and TBF using manufacturer's algorithms. Extra and intracellular fluids behave as resistive ® components and R is inversely proportional to fluid volume and therefore swelling (ECF) (5, 17).

Readily available ECG electrodes (Kendall CA610 diagnostic tab electrodes - reference code 31447793, Covidien, Mansfield, MA, USA) were utilised.

3.2.3 Data Collection

Bioimpedance triplicate measures, with one second intervals between each measure, were taken in two dressing conditions 1) no dressing or an open wound, and 2) new Acticoat^{TM} dressing. BIS measures were taken within 5 minutes of the dressing being applied. The time between the open wound and new Acticoat^{TM} dressing was recorded as this was unable to be standardised. The patient's weight and height, measured prior to the electrode placement, age and gender were input into the Impedimed instrument. All BIS measures were taken with the patient lying supine. Electrodes were placed over cleaned, intact skin in standard and alternate electrode whole body and limb segmental configurations unless precluded by wounds. If precluded by wounds that particular electrode configuration was not utilised. Due to the nature and presentation of moderate to large burns not all participants were able to have all electrode configurations assessed. Where feasible the measures were taken on the right side of the body unless precluded by wounds, then the left side was utilised. Electrodes remained in place between triplicate measures of each dressing condition. The researcher was blinded to the BIS measurements as only a file name was viewed and recorded.

3.2.4 Electrode Configurations

Standardised whole body and upper and lower limb segmental tetrapolar electrode placements were utilised as well as an alternative placement for each. Alternate electrode placements were used as burn wounds often preclude the placement in standardised positions and were determined based on the theory of equi-potentials (see Cornish et al (1999) for further explanation of equipotential points)(15). The different electrode configurations and actual placements were as follows (see Figure 3.1):

1) WBS: whole body standard tetrapolar placement

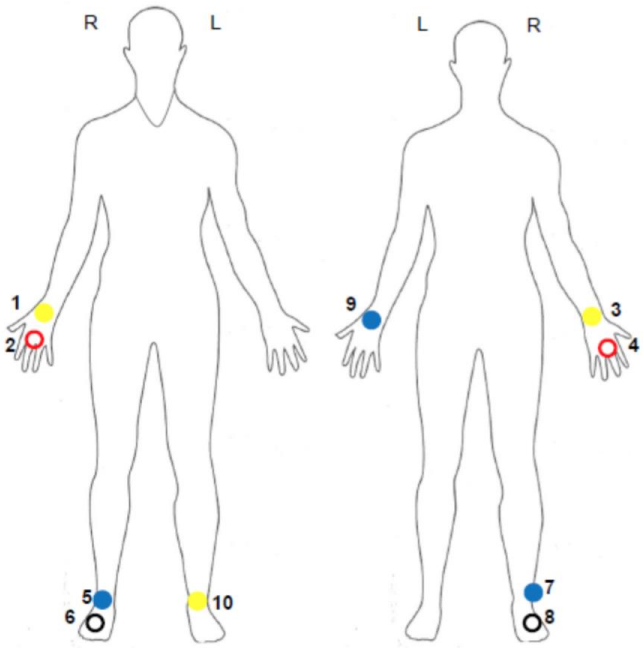
2) WBA: whole body alternate tetrapolar placement. Hand electrodes were placed on the volar surface of the hand and wrist, reflecting the standardised positions at the head of the third metacarpal and at the distal radio-ulnar joint. The foot electrodes were placed on the sole of the foot at the third metatarsophalangeal joint and anterior to the lateral aspect of the Achilles heel in line with the standard position.

3) ULS: upper limb standard tetrapolar placement. The right hand electrodes are as per the whole body standard tetrapolar placement, at the head of the third metacarpal and at the distal radio-ulnar joint on the dorsal surface. The left hand electrode was placed on the dorsal aspect of the distal radio-ulnar joint. The foot electrode was placed at the third metatarsophalangeal joint on the dorsal surface. As per Cornish, Jacobs et al.'s (1999) protocol (18).

4) ULA: upper limb alternate tetrapolar placement. The right side hand electrode placement was as per whole body alternate and the foot and left hand electrode placement remained in the standard position.

5) LLS: lower limb standard tetrapolar placement. The right hand electrode was placed at the head of the third metacarpal dorsally. The right foot electrode placements were at the third metatarsophalangeal joint and talocrural joint on the dorsal surface. The left foot electrode was placed at the dorsal talocrural joint. As per Cornish, Jacobs et al.'s (1999) protocol(15)

6) LLA: lower limb alternate tetrapolar placement. The right side foot electrode placement was as per whole body alternate and the left foot and hand electrode placement remained in the standard position.



Electrode Montage	Standard	Alternate
Whole Body	3,4,5,6	1,2,7,8
Upper Limb	3,4,6,9	1,2,8,9
Lower Limb	4,5,6,10	2,7,8,10

Figure 3.1: Electrode placement sites used for whole body, upper limb and lower limb BIS.

3.2.5 Ethics

This study was approved by the RPH Human Research Ethics Committee (EC 2011/028), and FSH (2014 106) Research Governance Committee and The University of Notre Dame, Australia Human Research Ethics Committee (014139F).

3.2.6 Statistical Analysis

All results were analysed using Stata statistical software, release 14 (StataCorp LP 2014, College Station, TX). Descriptive analyses were performed and are reported using the means and standard deviations (SD).

All BIS triplicate measures were used in the analysis. Multi-level mixed effects (MLME) linear regression was therefore utilised to determine whether electrode placement significantly affected the BIS variables. Whole body measurements had a separate model fitted for each of the raw variables R_0, $R_{i,}$ R_{inf} and calculated values (ECF, ICF, TBF). Segmental measures only had models fitted for each of the raw

variables, as the BIS algorithm is not applicable for segmental volume calculations. The MLME accounts for confounding variables thus limiting bias and it assumes that each variable in the regression is approximately normally distributed. To determine whether the whole body, upper and lower limb alternate electrode positions, in each dressing condition, were a valid measure of BIS variables a χ^2 post-estimation test was performed. It is a comparison of the difference of means as estimated by the regression coefficients determined by MLME linear regression. Results are reported as χ^2 statistic and a p-value of <0.05 was deemed significant for all analysis. The percentage difference between the alternate and standard electrode position was also calculated, in each dressing condition, whereby each BIS variable from the alternate electrode positions were expressed as a percentage change of the value obtained from the standard site. This assists with clinical application and meaning of the estimated BIS values. A percentage difference of greater than five percent was deemed clinically significant. There appears to be little consensus on an acceptable level of error in fluid assessment and monitoring clinically. Earthmann et al (2007) suggests a five percent error is tolerable (19).

3.3 Results

In line with the planned study timeframe and university milestones, the patient recruitment period was between December 2014 and February 2016. Twenty one patients were recruited on average 25 (SD = 11) hours post burn injury. There were two patients with burns < 15% TBSA who were fluid resuscitated were burnt while intoxicated and were considered clinically dehydrated. The final number of patients included in each electrode placement was: WBS (n=21), WBA (n=18), ULS (n=14), ULA (n=14), LLS (n=15), LLA (n=14). Other patient data is presented in Table 1.

Table 3.1: Patient data. Presented as means (standard deviations) ± range

%TBSA	Age (years)	Height (cm)	Weight (kg)	Time between open wound & new dressing (minutes)
24 (13) range 12-80	36 (13) range 18-63	172.2 (38.4)	77.4 (16.3)	66. 7 (31)

The means and confidence intervals for each of the BIS variables by electrode placement and dressing condition and the percentage difference between the alternate and standard electrode positions are presented in Table 3.1. The percentage difference between the alternate and standard electrode positions show a large variation across the variables (Table 3.2).

Table 3.2: Estimated BIS variable values for each electrode placement and dressing condition. Values presented as means (95% confidence intervals).

BIS Variable	Dressing Condition	Electrode Placement					
		WBS	**WBA**	**ULS**	**ULA**	**LLS**	**LLA**
R_0 (ohms)	Open	498.77 (467.17-530.37)	483.97 (451.79-516.15)	230.96 (196.89-265.03	231.79 (197.71-265.86)	268.77 (235.22-302.33)	254.69 (220.61-288.77)
	%difference		-2.97*		0.36*		-5.3
	Acticoat™	351.94 (295.56-408.32)	338.58 (281.33-395.83)	197.32 (134.23-260.40)	164.18 (103.24-225.12)	205.01 (145.75-264.27)	194.18 (134.14-254.21)
	%difference		-3.80*		-16.79		-5.30
R_i (ohms)	Open	1412.47 (1225.51-1599.42)	1353.01 (1164.26-1541.76	660.29 (465.29-855.29)	644.85 (449.83-839.87	791.23 (597.91-984.55)	722.00 (526.99-917.05)
	%difference		-4.21*		-2.34*		-8.75
	Acticoat™	715.75 (505.83-925.68)	679.88 (468.59-891.17)	439.66 (218.64-660.68)	355.05 (137.63-572.46)	497.52 (282.90-712.14)	425.37 (209.46-641.28)
	%difference		-5.02		-19.25		-14.51
R_{inf} (ohms)	Open	361.89 (337.57-386.20)	348.61 (324.02-373.20)	164.81 (139.28-190.35)	161.28 (135.74-186.81)	196.77 (171.49-222.05)	183.76 (158.22-209.29)
	%difference		-4.67*		-2.15*		-6.62
	Acticoat™	226.58 (183.50-269.67)	216.81 (173.16-260.46)	128.06 (80.55-175.57)	102.0139 (55.92-148.10)	135.01 (90.03-179.99)	122.57 (77.08-168.07)
	%difference		-4.32*		-20.34		-6.62
ECF (L)	Open	20.76 (17.56-23.97)	21.05 (17.80-24.29)	-	-	-	-
	%difference		1.40*				
	Acticoat™	34.77 (14.00-55.54)	35.54 (13.83-57.25)	-	-	-	-
	%difference		2.21*				
ICF (L)	Open	25.26 (21.62-28.91)	26.71 (23.03-30.40)	-	-	-	-
	%difference		5.74				
	Acticoat™	48.47 (27.74-69.21)	51.32 (29.83-72.82)	-	-	-	-
	%difference		5.88				
TBF (L)	Open	46.03 (39.67-52.38)	47.77 (41.36-54.19)	-	-	-	-
	%difference		3.78*				
	Acticoat™	83.16 (43.11-123.20)	86.66 (44.96-128.37	-	-	-	-
	%difference		4.21*				

R_0 = resistance at zero frequency, R_i = intracellular resistance, R_{inf} = resistance at infinite frequency, ECF = extracellular fluid, ICF = intracellular fluid, TBF = total body fluid, open = open wound, Acticoat™ = Acticoat™ and betadine compress dressing. % difference = % difference between alternate and standard electrode positions. * <5% in % difference. Electrode positions: WBS - whole body standard, WBA – whole body alternate, ULS - upper limb standard, ULA - upper limb alternate, LLS – lower limb standard, LLA – lower limb alternate.

The results of the post-estimation test analysis, χ^2, are shown in Table 3.3. The results show there is no statistically significant differences in the means of the BIS variables when comparing the standard and alternate electrode placements for the whole body, upper limb or lower limb segments (p = 0.097-0.96). This is true for any dressing condition.

Table 3.3: Difference in means comparison of standard and alternate electrode placement, in different dressing conditions for each of the BIS variables. Data presented as χ^2 (p-value).

Electrode placement	Dressing Condition	R_0 (ohms)	R_i (ohms)	R_{inf} (ohms)	ECF (L)	ICF (L)	TBF (L)
Whole Body	Open	1.28 (0.258)	1.10 (0.295)	2.76 (0.097)	0.06 (0.804)	1.59 (0.208)	0.88 (0.346)
	Acticoat™	0.40 (0.526)	0.49 (0.484)	0.44 (0.511)	0.004 (0.95)	0.06 (0.810)	0.02 (0.885)
Upper Limb	Open	0.00 (0.96)	0.06 (0.81)	0.15 (0.699)	-	-	-
	Acticoat™	1.61 (0.20)	1.79 (0.18)	2.00 (0.16)			
Lower Limb	Open	0.91 (0.34)	1.18 (0.28)	2.10 (0.15)	-	-	-
	Acticoat™	0.21 (0.65)	1.58 (0.21)	0.55 (0.46)			

Table 3.4: BIS measures in standard and alternate electrode placements in the healthy population.

BIS Variable	Electrode Placement					
	WBS	**WBA**	**ULS**	**ULA**	**LLS**	**LLA**
R_0 (ohms)	619.32	697.33	314.2	313.14	275.05	265.53
R_i (ohms)	1458.41	1388.82	797.35	816.89	656.60	587.72
Ri_{nf} (ohms)	428.19	416.61	220.68	221.43	191.62	180.55
ECF (L)	17.45	17.69				
ICF (L)	24.27	24.84				
TBF (L)	41.72	42.53				

R_0 = resistance at zero frequency, R_i = intracellular resistance, R_{inf} =resistance at infinite frequency, ECF = extracellular fluid, ICF = intracellular fluid, TBF = total body fluid. Electrode positions: WBS - whole body standard, WBA – whole body alternate, ULS - upper limb standard, ULA - upper limb alternate, LLS – lower limb standard, LLA – lower limb alternate.

3.4 Discussion

The results of this study demonstrate alternate whole body electrode placements (WBA) measure all resistances and generate ECF and TBF BIS variables comparable to whole body standardised placements (WBS) in burns greater than 12% TBSA within dressing conditions. Upper limb alternate segmental electrode placement also provides comparable BIS variable outputs with an open wound but not with an Acticoat^{TM} dressing.

Even though no statistical significant difference was found between standard and alternate electrode placements for all BIS variables, suggesting they are not different, consideration needs to be given to i) the percentage change between the two conditions and ii) the difference between the measured resistances and fluid volumes to determine clinically, if these differences are important. Each of these values needs to be considered in conjunction with one another, as there is the potential for volume over or understatement of up to 3.50L for TBF. A five percent difference was considered a clinically appropriate range for resistance and fluid volumes in the burns resuscitation environment. This could be in the order of ±1.25 L in an individual with 25 L of ICF, for example. This level was determined after considering the available literature and the need for maintenance of the intravascular volume with limited expansion of the extracellular volume in the burn resuscitation period. A volume change greater than 200 ml or a bioimpedance resistance ratio percentage change of greater than 10% is a suggested cut-off to identify secondary upper limb lymphoedema (20). In surgical gynaecological patients postoperative fluid overload was defined as being greater than 15% change in extracellular fluid (ECF) volume (determined by bioimpedance) from peri operative volumes (21).

Whole body electrode placements have a percentage change from WBS to WBA electrode placement for all BIS variables, except ICF, less than 5.02% in both dressing conditions. For R_0 and ECF (representative of oedema) the percentage difference between WBS and WBA electrode placement is $\leq$ 2.97% with an open wound and $\leq$ 3.80% with an Acticoat^{TM} dressing. These percentage differences are consistent with typical daily biological, intra-individual, within session variations, with multifrequency BIS, which ranges from 0.3-3% (as per manufacturers specifications) and up to 4% as reported by Kushner et al (22). Recently Pichonnaz et

al (2015) reported variations in some BIS variables below 5.6 %, may be considered measurement error (23). The actual estimated difference in R_0 and ECF between WBS and WBA electrode placements was -14.48 ohms and 245 ml in an open wound and -13.36 ohms and 770 ml in the Acticoat^{TM} dressing condition. Clinically, these may be considered acceptable changes in the acute burns resuscitation environment where rapid fluid shifts are occurring on the background of large resuscitation volumes e.g 13 354 ml (± 7386 ml) over 24 hours (24). This is in the realm of 500 ml of resuscitation fluid per hour. In gynaecological surgical cases an administered preoperative IV fluid volume of 1.9 L over 154 minutes resulted in an increase in ECF of 0.8 L (± 0.8 L), TBF of 1 L (± 1.4 L) and a stable ICF as measured by BIS (21). For TBF the volume difference between WBS and WBA were 1.74 L and 3.50 L for an open wound (no dressing) and Acticoat^{TM} dressing condition respectively. These values are less than a five percent difference, however a change from 1.74 L to 3.50 L between standard and alternate electrode placement in the Acticoat^{TM} condition is too large to be acceptable, potentially causing a patient to be under-resuscitated if alternate electrode positions were used. This suggests alternate electrode positions cannot be relied upon in the Acticoat^{TM} condition to monitor TBF volumes. In contrast, R_{inf} the equivalent raw variable of TBF, mean difference is 13.28 ohms in an open wound and 9.77 ohms in an Acticoat^{TM} dressing. These are considered acceptable when the mean R_{inf} is 348-361 ohms and 216-226 ohms in the respective dressing conditions.

Although there was no statistically significant difference between whole body standard and alternate electrode placement for ICF the percentage difference was 5.74% and 5.88%, with the greatest change in the Acticoat^{TM} dressing condition. This is above the normal biological variation range, accepted 5 percent error and in the order of 1.45 L and 2.85 L difference in volume between WBS and WBA for the open wound and Acticoat^{TM} dressing condition respectively. This variation is considered too great to be used clinically, as it could lead to under or over resuscitation of a patient. Yet the corresponding resistance (R_i) percentage difference was 57.66 ohms and 34.51 ohms respectively. These values are less than 5.02% difference and also considered insignificant on the background of whole body R_i values of 1350-1412 ohms (open wound) and 680-715 ohms (Acticoat$^{TM)}$. The WBA electrode placement

was the same utilised in Grisbrook et al's (2015) study (25). However, they found all BIS fluid volumes to be significantly overestimated in healthy individuals.

Whole body alternate electrode positions are comparable to that of the standard positioning for measuring BIS resistance variables, ECF and TBF (within the specified dressing conditions) but not ICF. Clinically, whole body BIS resistance values can be used to monitor changes in inter-compartmental fluid volumes and this is supported in the literature. Ward et al (2006) reported raw resistance values could be used as a surrogate index of volume due to their inverse relationship (26). It has also been suggested in the literature that raw BIS data may prove to be more clinically useful as it removes the need for predictive equations (27). Further support for the use of whole body BIS in daily monitoring of fluid volumes is the comparison of our BIS measures to normative values (Figure 3.4) (25). This study utilised the same alternate electrode placement as Grisbrook et al (2015). Considering the average time post burn was 25 hours, with a potential fluid resuscitation volume of up to ~ four litres over this period the results of the BIS measures seem reasonable. i.e the difference in standard BIS fluid volume measures between burns and healthy populations are ECF 3.31 L, ICF 0.99 L, TBF 4.31 L (Table 3.4). The validity of BIS in its ability to measure fluid inter-compartmental volumes in major burns however is yet to be determined. Future research should therefore explore this.

In the upper limb electrode positions however, there were large percentage differences (range 16.79-20.34%) in the Acticoat^{TM} dressing condition compared to an open wound (range 0.36-2.34%). There was however no statistical significance difference found between ULS and ULA electrode placements in χ^2 test ($p = 0.16$-0.2) of the mean BIS values for each dressing condition. Upper limb alternate electrode placement can be utilised if wounds preclude the use of standard placements in the open wound as they give comparable measures. However, the large mean percentage change between ULS and ULA with Acticoat^{TM} insitu does not support the use of ULA in this dressing condition. Grisbrook et al (2015) found placement of electrodes on the ventral surface of the hand and wrist for upper limb segmental measures were valid alternatives to the standard placement in the healthy population (no dressings insitu) (25).

No statistically significant differences were found between the LLS and LLA electrode placements, but they too also had higher than accepted intra-individual

biological variations in their mean percentage differences of the resistance variables (range 5.3 – 14.56%). Resistance at zero frequency had the lowest values (5.30% for both an open wound and Acticoat^{TM} dressing) with R_i having the greatest values. Such large percentage differences render LLA electrode placement unsuitable to be used if wounds preclude the placement of standardised lower limb electrode placements. This again, is consistent with the findings of Grisbrook et al (2015) in terms of potential clinical utility, although they found a statistical difference between LLS and LLA electrode placements (25). Limb segmental BIS measures provide only raw resistance variables, as they require a separate algorithm to calculate fluid volumes. The segmental measures were included in this research to determine whether they are a potential alternative to whole body BIS but further research is required for clinically meaningful application.

A statistical significant difference was not found between any of the standard and alternate electrode placements however the percentage difference was deemed clinically significant for the aforementioned whole body fluid variables ICF, TBF and all lower limb resistances with and without Acticoat^{TM}, and all upper limb resistances with Acticoat^{TM}. This could be explained by the potential risk of type two error in the study due to the relative small sample size in each electrode placement group (n=14-18) i.e. failing to reject the null hypothesis that there is a difference between standard and alternate electrode placement. Another possible reason for the larger differences in the BIS variables, between standard and alternate positions in the Acticoat^{TM} dressing condition, is the age of the electrodes. The electrodes were kept in place between the open and new dressing condition to reduce the risk of electrode mismatch placement measurement error, which can be in the order of 4% (14). However, the electrochemical properties of the electrodes change with time (28) and resistivity decreases with moisture thus decreasing the resistance, however the magnitude of influence is unknown (29, 30). Burns patients stress levels and skin temperature will often increase during a dressing change and with dressings in place. The palms and soles of the feet (location of alternate electrode positions) have the highest density of sweat glands in the body (31). Therefore the resistance measured in the alternate electrode placements, especially of the hand, may be further decreased, increasing the percentage difference between BIS resistance values in the Acticoat^{TM} dressing compared to the open wound.

3.4.1 Future Research

To make progress in the field new alternative electrode placements need to be investigated to ascertain whether they are comparable to standardised placement for all BIS variable outputs. It is evident the Acticoat™ exaggerates the differences between the standard and alternate electrode positions but also between the open wound and Acticoat™ dressing condition. Future studies should therefore examine alternate electrode placements in the Acticoat™ condition. An Acticoat™ BIS calculator to adjust for the Acticoat™ effect in moderate to large burns (unpublished data) is currently being developed. To further enhance the clinical applicability of BIS in burns, studies investigating alternate electrode placements in other dressing conditions are also warranted.

3.5 Conclusion

This study determined whole body alternate electrode placements are a feasible alternative when wounds preclude the use of standardised placement for monitoring R_0, R_i, R_{inf} and ECF within dressing conditions in burns >12% TBSA. Further research is required to establish the best alternate electrode placements to measure all BIS variables in moderate to large burns.

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Chapter 4 An Objective Measure For The Assessment And Management Of Fluid Shifts In Acute Major Burns

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The PhD candidate, Pippa Kenworthy accounted for 85% of the intellectual property associated with the final manuscript. Collectively the remaining authors contributed 15%.

Foreword

The preceding study determined whole body and upper limb segmental resistance variables and whole body ECF and TBF volumes only in alternate electrode positions were interchangeable with standardised positions in specified dressing conditions. Hence providing a substitute when wounds preclude standardised electrode placement to enable monitoring of fluid volume change in acute major burns. Following on, applying these findings, the next study investigated the reliability and validity of BIS as a measure of fluid shifts in acute major burns, and the impact of dressings on BIS measures. Bioimpedance spectroscopy has been shown to be sensitive measure of oedema volume change in large burns. It is reliable in burns less than 30% TBSA across different dressing conditions but it is yet to be validated as a method of fluid shift over time in moderate to large burns. Acticoat™, a nanocrystalline silver dressing used in the first 48 hours of care in the BSWA, has been demonstrated to effect BIS measures in burns not receiving fluid resuscitation however the effect on those receiving fluid resuscitation is not known.

4.1 Introduction

Large fluid shifts and local and distant tissue swelling are features of burn injuries. Swelling hampers burn wound healing and the volume created is directly related to the size and depth of the burn (1). Major burns greater than 15-20% total body surface area (TBSA) with a depth of partial to full thickness result in both a local and systemic inflammatory response (2, 3). This can be a life threatening scenario which requires formal fluid resuscitation. Acute burn fluid resuscitation is vital in decreasing patient morbidity and mortality in the first 24-48 hours of injury but can contribute to already large amounts of oedema (4).

Despite the importance of fluid resuscitation in the early management of traumatic burn injuries, there is currently no single, simple, non-invasive and accurate outcome measure which can assist clinicians to titrate fluid volumes in acute burns or monitor the effect of treatments on swelling. Thus, the objective, timely adjustment of fluid resuscitation is challenging, particularly when patients are not supported by critical care and invasive monitoring. This research investigates the accuracy of bioimpedance spectroscopy (BIS) in monitoring whole body fluid volume and oedema change in moderate to large acute burns.

There has been little advancement in the area of burn fluid resuscitation over the last 30 years (4) and in recent times there has been a trend to over resuscitate patients (5, 6), necessitating a descriptor known as *fluid creep*. Excess fluid can contribute to burn wound progression, lead to complications such as peripheral and abdominal compartment syndromes, pulmonary oedema and peripheral tissue oedema. Any one or a combination of these will affect patient recovery, increase medical costs and is likely to increase patient length of stay (3, 7-10).

Fluid resuscitation formulas such as the Parkland and Brookes are used to instigate intravenous (IV) fluid rates but are guidelines only and fluid must then be titrated according to particular endpoints of resuscitation (11-13). The most commonly used outcome measure for fluid therapy is urine output, with the aim to maintain a rate of 30-50ml per hour for an average sized man while preserving haemodynamic properties such as oxygen saturation and blood pressure (5, 14). There are other

objective measures to guide volume titration however they are invasive and not without limitations (6, 14, 15). oedema

Bioimpedance spectroscopy has historically been used in healthy populations to measure body composition. However in the last 20 years it has gained increasing popularity in clinical populations and is now commonly used to measure arm lymphoedema post breast surgery (16) and dry weight in haemodialysis patients (17, 18). Bioimpedance spectroscopy has demonstrated sensitivity, high reliability (repeatability) of measures in a number of clinical areas (19). The method has also been validated (determined credible) in both healthy and clinical populations against MRI and bromide and potassium dilution techniques, which are considered gold standard in the assessment of fluid compartment volumes and lean body mass (LBM) (20-23). It can investigate the body's physiological parameters such as extracellular fluid (ECF), intracellular fluid (ICF) and total body fluid (TBF). It achieves this by passing a small alternating current, over a number of frequencies (4-1000 kHz), through the tissues and fluid compartments of the body via electrodes on intact skin. It provides instantaneous measures of resistance (R) and reactance (capacitive resistance (Xc)). Resistance is the opposition to flow of an electric current, is reflective of the body's water compartments and is inversely proportional to fluid volume and therefore oedema (24, 25). Capacitance is the delay in the passage of current through the cell membranes and tissue interfaces (25). The current flow is frequency (Hz) dependent and varies according to the composition of the body (26). Resistances at zero and infinite frequencies (considered ideal measurement frequencies) are estimated utilising the Cole-Cole plot embedded in the BIS software, due the constraints of using a direct or very high frequency alternating current in humans (27). The resistance at zero (R_0) and infinite (R_{inf}) frequencies (25) are representative of extracellular fluid (ECF) and total body fluid (TBF) respectively. Resistance (R_i) of the intracellular fluid (ICF) is extrapolated using the other raw variable data. At low frequencies the current can penetrate the ECF only and at high frequencies it passes through both the ECF and ICF measuring TBF.

The ability of BIS to quantify individual body fluid compartments, the ease of use and non-invasive nature has led to a small number of papers examining its use in the burn population. Miller et al (1999) and Zdolsek et al (1998) were able to determine

the development of oedema post burn injury but each study lacked power and neither was able to provide statistical conclusions regarding the reliability of BIS in the burns populace. In 2009 Edgar et al demonstrated whole body bioimpedance spectroscopy was a reliable means of quantifying real time oedema shifts in patients with burns less than 30% TBSA across numerous dressing conditions (28). However the study only had 6 participants with burns greater than 15% TBSA and was therefore inconclusive in this subset of patients. Further each study utilised standard whole body electrode positions only and it is unknown whether alternate electrode positions, for both whole body and limb segmental BIS, are reliable in this particular population. Grisbrook et al (2015) investigated whether alternate electrode configuration BIS measurements were interchangeable with standard electrode configurations in the healthy population but reliability was not determined (29). In Edgar et al's (2009) study it was also apparent the dressing condition affected the sensitivity of the BIS results. Bioimpedance measures were found to be less sensitive in older dressings (> 8 hours old) than in an open wound or new dressing condition.

Dressing-type may pose a further challenge in the assessment of fluid shifts by BIS. Acticoat™ (Smith & Nephew) is an antimicrobial dressing, composed of nanocrystalline silver particles (30). It is the standard dressing used in the first 48 hours of burn care, and as indicated after, in the Burn Service of Western Australia (BSWA). Understanding that BIS measures the resistance of the body's tissues and inter-compartmental fluid volumes by introducing a low amplitude electrical current into the body, it would not be unexpected that Acticoat™ may affect the BIS measures. Silver is a highly conductive material, and such dressings release ionic silver species and are applied in a wet condition. Both the silver ions and wet condition would therefore be expected to reduce the BIS resistance measured, thus potentially limiting the use of monitoring fluid shifts with BIS in acute burns patients.

To extend Edgar et al's (2009) reliability study and on the premise that BIS can reliably quantify tissue fluid, it was hypothesized BIS would provide a method for real time accurate measures of fluid shifts in the acute major burn. The study aimed to a) examine the reliability with respect to dressing condition and electrode position, b) investigate the influence of Acticoat™ on BIS variable outputs and c) determine

the validity of whole body BIS to assess net fluid shift in the presence of moderate to major burns, greater than 15% TBSA.

4.2 Methods

4.2.1 Participants

An observational longitudinal cohort study was conducted from December 2014 to February 2016. Patients were recruited into the study if they were: over eighteen years old, receiving formal fluid resuscitation had a flame and/or scald burn and the injury was less than 48 hours old. The BSWA medical team instigates fluid resuscitation for partial to deep thickness burns greater than 15% TBSA (modified however based on each individuals clinical presentation and nutritional status at admission) and uses Ringer's Lactate (crystalloid) solution with volumes initially determined by the modified Parkland's formula. Fluid volumes were titrated to maintain an adequate urine output of 0.5-1.0ml/kg/hr for the first 36-48 hours after burn injury. Participants were excluded from the research if they had: hand and/or feet burns precluding placement of standard whole body electrode placement, body mass index (BMI) ≤ 15 and ≥ 40 kg/m^2 (manufacturer's guidelines) and if they met Impedimed SFB7 (ImpediMed, Brisbane, Queensland, Australia) manufacturer's contraindications which includes pregnant or breast-feeding patients, patients with surgical implants, cardiac pacemakers and/or are on electronic life support devices (ventilated patients).

Burn inpatients were recruited initially from the Burn Unit at Royal Perth Hospital (RPH) and then at Fiona Stanley Hospital (FSH) due to the transition of the adult care of the BSWA to the new Fiona Stanley Hospital. There was no change to the study protocol or equipment used in the study.

4.2.2 Equipment

The ImpediMed SFB7 was used to collect whole body and segmental BIS measures (Figure 4.1). The calculated fluid volumes are stable when the subject's BMI is > 15 kg/m^2 (as per the manufacturer).

The BIS equipment measures both raw resistance variables and derived fluid distribution values such as whole body ECF, ICF, and TBF using manufacturer's algorithms. It achieves this by applying 256 discrete current frequencies (4-1000 Hz) through the body. Extra and intracellular fluids behave as resistive (R) components and R is inversely proportional to fluid volume (26, 31).

Diagnostic tab electrodes, Kendall CA610 (reference code 31447793, Covidien, Mansfield, MA, USA), were utilised.

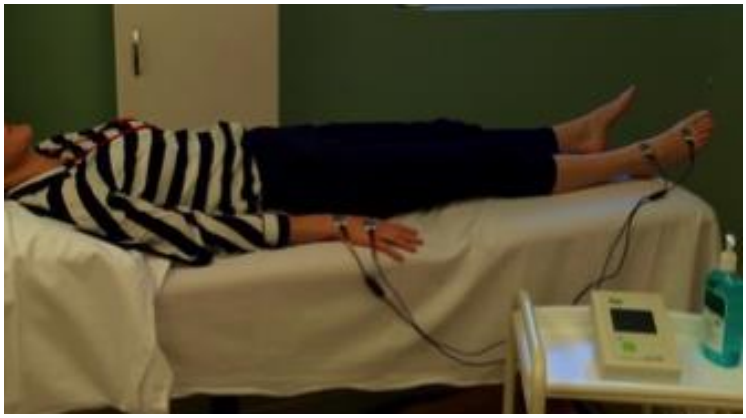

Figure 4.1: Bioimpedance spectroscopy: standard whole body electrode positions

4.2.3 Procedures

Firstly, the patient's weight and height was measured and input into the Impedimed instrument along with their age and gender. All BIS measures were taken using manufacturer's recommended and standardised positions with the patient lying supine and with arms and legs abducted away from the body. BIS electrodes were placed over intact, cleaned skin (using alcohol swabs).

4.2.3.1 Electrode Configurations

Standardised tetrapolar electrode placements (EP) were utilised (25, 32) and alternate electrode configurations were placed based on the theory of equi-potentials (see Cornish et al (1999) for further details of equipotential points) (32) and were placed as per Grisbrook et al (2015). Electrodes were placed on intact skin only. Participants with bilateral hand or foot injuries which precluded the application of standardised electrode placements were excluded. Bioimpedance measures were taken on the right

side of the body unless precluded by wounds, then the left side was utilised. The location of their wounds determined whether all other electrode placements (segmental) could be used and measured.

BIS measures were taken in triplicate in an open wound (time point 0 (T0)) and in the new Acticoat^{TM} dressing condition at five (5) half hour intervals (T1-5) after the baseline measure i.e. five measures in total (Figure 4.2). The time between T0 and T1 was recorded, as this was unable to be standardised. Standard and alternate whole body, upper limb segmental and lower limb segmental BIS measures were taken at at T0-T1. Standard whole body EP's only were utilised at T2-T5 (Figure 4.2). Burn wounds often prevent electrodes being applied in the standard position, therefore alternative whole body and limb segment electrode positions were utilised as able at T0-T1 and their reliability investigated. The data to determine the validity of alternate electrode placement has been analysed separately (33). The segmental measures were included in the reliability analysis only. The effect of Acticoat^{TM} on whole body BIS results was determined from T0-T1 BIS readings. Electrodes remained in situ between triplicate measures where possible, unless prohibited by dressing changes or adhesive loss.

Net fluid shift was recorded between each time point (T1-5), in conjunction with the BIS measures. Net fluid shift was calculated by subtracting urine output and other bodily fluid output recorded (e.g. emesis) from fluid intake (IV and oral fluids and food).

The researcher was blinded to all BIS measurements as only a file name was viewed and recorded, not the actual BIS values.

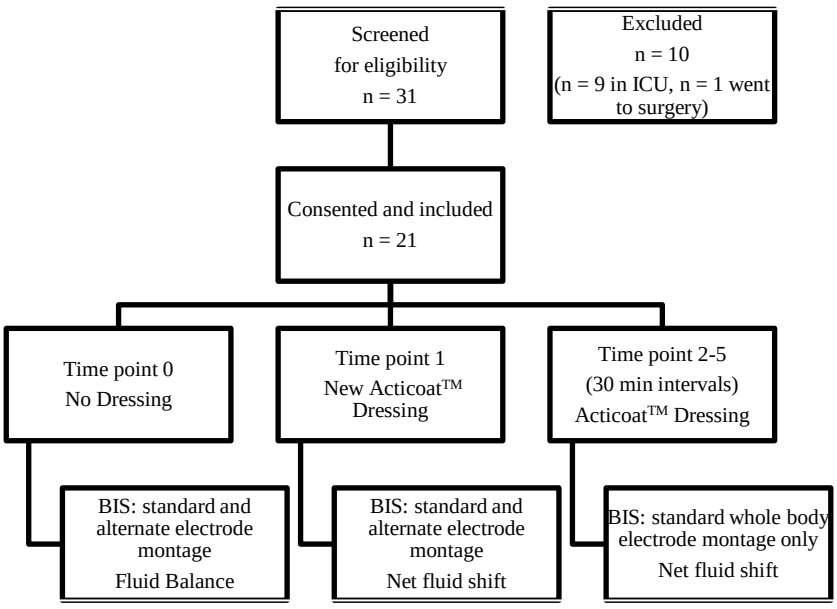

Figure 4.2: Consort Diagram-Flow diagram of data collection process

4.2.4 Ethics

This study was approved by RPH Ethics Committee (EC 2011/028), FSH Research Governance Committee (2014 106) and The University of Notre Dame, Australia Human Research Ethics Committee (014139F).

4.2.5 Data Analysis

Stata statistical software, release 14 (StataCorp LP 2014, College Station, TX), was utilised to analyse all results. Descriptive analyses were performed and are reported using the means and standard deviations (SD).

4.2.5.1 Reliability

A three level nested mixed effects linear regression was performed to examine the reliability of the BIS triplicate measures, taking into account random effects of confounders of electrode position, time and dressing condition. The multilevel mixed effects (MLME) linear regression also explored whether there was a significant within-session difference between the triplicate measures for each of the BIS variables. Reliability is presented as the intra-class correlation coefficient (ICC) (acceptable, 0.75-0.89, excellent ≥ 0.9) (34), variance indicated by 95% confidence

intervals (CI) and systematic bias between within session trial measures ($p < 0.05$ considered significant). All BIS triplicate measures were used in the analysis.

Analysis was completed using the MLME model as it can account for random effects from individuals and responses within individuals (35). It is a robust method providing hierarchical analysis, adjusting for nested observations of measures for each individual and gives the most precise and least biased estimates of treatment effects. Prior to interpreting the results of the MLME, several assumptions were evaluated, confirming that each variable in the regression was approximately normally distributed.

4.2.5.2 Factors influencing BIS readings

The effect of dressing condition, %TBSA and initial TBF on the BIS whole body variables only was determined by MLME linear regression. A separate model was performed for each BIS variable. The interaction between Acticoat™ and %TBSA and their influence on the BIS variables was also examined. The whole body standard and alternate electrode placement BIS variable outputs were grouped together for use in the analysis for the effect of Acticoat™ and %TBSA. Time point 0 (open wound) and TP1 (new Acticoat™ dressing) were used only.

4.2.5.3 Validity

Validity was determined using a series of MLME linear regression models including the data with the Acticoat™ dressing condition only, and whole body standard electrode placement (T1-T5) and alternate electrode placement (T1) only. The final model was produced by completing step-wise, backward elimination of predictor variables on each of the dependent BIS variables. The final model included %TBSA, time, net fluid shift and initial TBF volume. Initial TBF volume was derived from the mean of the TBF measured with an open wound using standard tetrapolar whole body electrode placement as single frequency BIA has been shown to measure TBF accurately in burns patients with no dressings (36). This provided a baseline total body volume (L). A correlation matrix was performed to determine the relationship between initial TBF, weight and height and the skewness-kurtosis test demonstrated that they were each normally distributed.

Change scores or calculated difference of the BIS variables between time points (e.g. R_0 at T2 - R_0 at T1) were not used in the validity analysis, as the calculation of a change score requires measurement of the outcome twice and in practice it is proposed that it is more efficient to use a (single) change from baseline measurement to derive outcomes. In addition by not analysing change (difference) data, the additive effect of the random errors is potentially reduced (37).

4.2.5.4 Calculator

A calculator was developed to estimate the net fluid shift between consecutive BIS measures, when an Acticoat™ dressing is in place. Algorithms, for calculation of estimated fluid volumes were developed incorporating the significant and influential variables (on BIS variables) from the MLME models.

4.3 Results

Twenty one patients, 7 females and 14 males, were recruited post burn injury. One patient had an incomplete set of fluid recordings and 2 patients only had repeated measures completed 4 times in the new Acticoat™ dressing condition. The mean net fluid shift (SD) at each time point, separated by ~30 minutes for T1-T5, were as follows, T1 174.72 ml (533.18), T2 189.15 ml (164.23), T3 204.00 ml (135.37), T4 141.48 (253.25) and T5 123.20 (114.33). The average time between T0 –T1 (SD) was 67 minutes (31). The mean TBF (SD) of patients on initial assessment was 46.06 L (9.71). Other patient data are presented in Table 4.1.

Table 4.1: Patient data (n=21).

%TBSA	Age (years)	Recruitment post burn injury (hrs)	Height (cm)	Weight (kg)
24 (13) Range 12-80	36.4 (13.5)	25 (11)	172.2	77.4 (16.3)

Values presented as means (SD) ± range

4.3.1 Reliability

BIS triplicate measures were reliable within any electrode position, dressing condition and over time. Table 4.2 presents that BIS was a reliable measure in all circumstances, as confirmed by the ICC's. There were no significant differences between the estimated means of within session triplicate trial measures for each of the BIS variables (ie no systematic bias) (Table 4.2). Final numbers included in each EP analysis were WBS (n=21), WBA (n=18), ULS (n=14), ULA (n=14), LLS (n=15), LLA (n=14).

Table 4.2: BIS Reliability

BIS Variable	ICC (95% CI)	BIS trial number*	BIS measure Coefficient (95% CI)	p-value
R_0	0.999 (0.999-0.999)	2	-0.07 (-0.68-0.54)	0.83
		3	-0.06 (-0.68-0.55)	0.84
R_i	0.999 (0.998-0.999)	2	0.41 (-1.90-2.71)	0.73
		3	2.06 (-0.24-4.37)	0.80
R_{inf}	0.9996(0.999-0.999)	2	0.01 (-0.30-0.32)	0.94
		3	0.07 (-0.24-0.38)	0.66
ECF	0.999 (0.998-0.999)	2	0.03 (-0.17-0.22)	0.78
		3	0.12 (-0.07-0.32)	0.22
ICF	0.997 (0.996-0.998)	2	-0.12 (-0.46-0.22)	0.49
		3	-0.26 (-0.61-0.08)	0.13
TBF	0.999 (0.999-0.999)	2	-0.09 (-0.38-0.20)	0.53
		3	-0.14 (-0.43-0.15)	0.33

ICC = intraclass correlation coefficient, R_0 = resistance at zero frequency, R_i = intracellular resistance, R_{inf} =resistance at infinite frequency, ECF = extracellular fluid, ICF = intracellular fluid, TBF = total body fluid. *Each BIS measure coefficient is in reference to measure 1 of the triplicate measures.

The means and CI for each of the BIS variables for the standard whole body electrode placement and time point are presented in Table 4.3.

Table 4.3: BIS variable values for the standard whole body electrode placement and time point. Values presented as means (confidence intervals).

BIS Variable At WBS	Time Point					
	T0	T1	T2	T3	T4	T5
R_0 (ohms)	498.77 (467.17-530.37)	351.94 (295.56-408.32)	366.70 (314.94-418.45)	371.18 (319.50-422.86)	371.76 (322.20-422.33)	401.01 (348.18-45384)
R_i (ohms)	1412.47 (1225.51-1599.42)	715.75 (505.83-925.68)	715.51 (536.09 894.93)	721.81 (546.31-897.31)	713.41 (541.38-885.44)	798.52 (611.02-986.02)
R_{inf} (ohms)	361.89 (337.57-386.20)	226.58 (183.50-269.67)	234.35 (195.19-273.52)	237.45 (198.23-276.67)	238.65 (200.24-277.06)	261.95 (220.50-303.40)
ECF (L)	20.76 (17.56-23.97)	34.77 (14.00-55.54)	32.50 (13.22-51.78)	31.93 (14.21-49.66)	31.50 (15.07-47.92)	24.84 (10.44-39.25)
ICF (L)	25.26 (21.62-28.91)	48.47 (27.74-69.21)	46.97 (27.11-66.83)	46.71 (27.15-66.27)	46.18 (27.20-65.16)	37.80 (21.38-54.23)
TBF (L)	46.03 (39.67-52.38)	83.16 (43.11-123.20)	79.48 (41.84-117.12)	78.53 (42.85-114.20)	77.65 (43.18-112.11)	62.65 (33.67-91.63)

WBS = standard whole body electrode position, R_0 = resistance at zero frequency, R_i = intracellular resistance, R_{inf} =resistance at infinite frequency, ECF = extracellular fluid, ICF = intracellular fluid, TBF = total body fluid. T0 = initial BIS measurement with no dressing, TBSA = total body surface area, T1= first BIS measure with new Acticoat™ dressing, T2-5= BIS measures taken at half hourly intervals. intervals. Values presented as means (confidence intervals).

4.3.2 Factors influencing BIS readings

The regression analysis demonstrated Acticoat™ had a significant effect on the raw variables R_i and R_{inf} (but not R_0) and on all the calculated variables (ECF, ICF, TBF) in whole body BIS (Table 4.4). The resistance variables reduced between 182.22 and 23.87 ohms for R_i and R_{inf} and the calculated volumes were increased by 31.00 – 67.23 L when an Acticoat™ dressing was in place, compared to the open wound condition.

There was no evidence of an effect of TBSA on any of the BIS variables (Table 4.4). However there was a statistically significant interaction ($p < 0.01$) between TBSA and Acticoat™ for all BIS variables, raw and calculated. When an Acticoat™ dressing was in place and for every 1% increase in TBSA R_0 decreased by 4.68 ohms, R_i by 17.98 ohms and R_{inf} by 3.96 ohms. This results in a divergence away from the open wound R values as TBSA% increases. Extracellular fluid, ICF and

TBF volumes all increased with greater TBSA when an Acticoat^{TM} dressing was in place also resulting in divergence away from the open wound fluid volumes as TBSA increased (Table 4.4).

As expected, there was a strong positive correlation between initial TBF and weight, with a correlation coefficient (r) of 0.83 ($p < 0.01$). There was also a moderate positive correlation between initial TBF and height, $r = 0.67$ ($p < 0.01$). Initial TBF was therefore included in the model, and height omitted, to reduce collinearity. Initial TBF was included in preference to BMI as it was determined to be a more robust indicator of a person's size as the random error was reduced when compared to BMI (as it is one variable compared to two (height and weight)). Initial TBF is significantly associated with all BIS variables. For every 1 L increase in initial TBF R_0 decreased by 5.71 ohms ($p < 0.01$), R_i decreased by 32.52 ohms ($p < 0.01$) and R_{inf} decreased by 5.30 ohms ($p < 0.01$). All estimated fluid volumes increased (ECF 0.93 L, ICF 1.08 L, TBF 2.02 L) with every 1 L increase in initial TBF.

Algorithms were developed to correct for the effect of Acticoat^{TM} for the BIS variables. They are as follows:

Corrected ECF = measured ECF with Acticoat dressing – (-59.02 + (time since dressing applied*1.38) + (initial measured ECF*2.69))

Corrected ICF = measured ICF with Acticoat dressing – (-79.26 + (time since dressing applied*-0.0006) + (%TBSA*1.85) + (initial measured ICF*3.088918))

Table 4.4: Predictor variable effects on whole body BIS variables for determining the effect of Acticoat™

BIS variable	Covariate	Co-efficient	Confidence intervals		p-value
			Lower	Upper	
R_0	Acticoat™	-17.42	-39.35	4.52	0.12
	% TBSA	-1.07	-2.75	0.61	0.21
	Acticoat™#% TBSA	-4.68	-5.37	-3.98	<0.01*
	Initial TBF (L)	-5.71	-8.32	-3.09	<0.01*
R_i	Acticoat™	-182.22	-265.27	-99.16	<0.01*
	% TBSA	6.50	-3.45	16.46	0.20
	Acticoat™#% TBSA	-17.98	-20.61	-15.36	<0.01*
	Initial TBF (L)	-32.52	-48.16	-16.87	<0.01*
R_{inf}	Acticoat™	-23.87	-38.57	-9.17	<0.01*
	% TBSA	-0.01	-1.33	1.32	0.99
	Acticoat™#% TBSA	-3.96	-4.42	-3.49	<0.01*
	Initial TBF (L)	-5.30	-7.37	-3.23	<0.01*
ECF	Acticoat™	-36.23	-41.91	-30.55	<0.01*
	% TBSA	-0.04	-0.31	0.23	0.76
	Acticoat™#% TBSA	1.86	1.68	2.04	<0.01*
	Initial TBF (L)	0.93	0.53	1.33	<0.01*
ICF	Acticoat™	-31.00	-36.07	-25.92	<0.01*
	% TBSA	-0.15	-0.36	0.07	0.18
	Acticoat™#% TBSA	2.01	1.85	2.17	<0.01*
	Initial TBF (L)	1.08	0.77	1.40	<0.01*
TBF	Acticoat™	-67.23	-77.13	-57.32	<0.01*
	% TBSA	-0.19	-0.63	0.25	0.40
	Acticoat™#% TBSA	3.87	3.55	4.18	<0.01*
	Initial TBF (L)	2.02	1.36	2.67	<0.01*

R_0 = resistance at zero frequency (ohms), R_i = intracellular resistance (ohms), R_{inf} = resistance at infinite frequency (ohms), ECF = extracellular fluid (L), ICF = intracellular fluid (L), TBF = total body fluid (L), TBSA = total body surface area, # = interaction term, *p= <0.05. Acticoat™ is in reference to an open wound

4.3.3 Validity

BIS resistance and fluid volume variables were analysed to determine BIS validity. The MLME linear regression univariate analysis, in the Acticoat™ dressing condition only, showed R_0, R_i and R_{inf} significantly changed with time (Table 4.5). Compared to T1 (new Acticoat™ dressing), for every minute increase in time, R_0 decreased 0.40 ohms ($p < 0.01$), R_i decreased 2.51 ohms ($p < 0.01$) and R_{inf} decreased 0.40 ohms ($p < 0.01$). The BIS calculated fluid volumes ICF and TBF were also

significantly associated with time, increasing by 60 ml and 20 ml for every minute increase in time ($p < 0.01$). ECF was not significantly associated with time.

The regression analyses demonstrated all resistance values significantly decreased with increasing net fluid volume in a linear relationship (Table 4.5, Figure 4.3 A). Net fluid volume was significantly associated with ICF and TBF BIS fluid volume change, increasing with increasing net fluid shift (Figure 4.3 B). All BIS variables were significantly associated with % TBSA. For every 1% increase in TBSA R_0 decreased 5.09 ohms, R_i decreased 8.85 ohms and R_{inf} decreased 3.25 ohms. Fluid volumes increased between 1.20 – 2.77 L with every 1% increase in TBSA ($p < 0.01$) (Table 4.5).

Two individuals who had large negative fluid shifts >850 ml across a single time point were removed from the analysis after step wise analysis found they significantly altered the results of the final model Leaving these patients in the analysis would have resulted in a non-homogenous sample. It appears a large loss of fluid volume compromises the interpretation of BIS measures. Both patients suffered loss of large volumes of ionic fluid due to emesis which likely altered the measured BIS resistance (27).

When a patient's initial TBF increased by 1 L R_0 decreased 5.78 ohms ($p <0.01$), R_i decreased 28.79 ohms ($p <0.01$) and R_{inf} decreased 5.31 ohms ($p <0.01$).

Table 4.5: Univariate analysis of variable correlation on whole body BIS measures

BIS variable	Covariate	Co-efficient	Confidence intervals		p-value
			Lower	Upper	
R_0	Time (minutes)	-0.40	-0.54	-0.27	<0.01*
	% TBSA	-5.09	-7.08	-3.10	<0.01*
	Net fluid shift (ml)	-0.05	-0.07	-0.02	<0.01*
	Initial TBF (L)	-5.78	-8.95	-2.61	<0.01*
R_i	Time (minutes)	-2.51	-3.09	-1.92	<0.01*
	% TBSA	-8.85	-16.98	-0.74	0.03*
	Net fluid shift (ml)	-0.25	-0.36	-0.15	<0.01*
	Initial TBF (L)	-28.79	-41.74	-15.84	<0.01*
R_{inf}	Time (minutes)	-0.40	-0.51	-0.28	<0.01*
	% TBSA	-3.25	-4.69	-1.81	<0.01*
	Net fluid shift (ml)	-0.05	-0.07	-0.03	<0.01*
	Initial TBF (L)	-5.38	-7.68	-3.07	<0.01*
ECF	Time (minutes)	0.02	-0.01	0.05	0.15
	% TBSA	1.40	0.99	1.80	<0.01*
	Net fluid shift (ml)	0.01	-0.001	0.01	0.09
	Initial TBF (L)	1.20	0.56	1.85	<0.01*
ICF	Time (minutes)	0.06	0.03	0.10	<0.01*
	% TBSA	1.52	1.17	1.88	<0.01*
	Net fluid shift (ml)	0.01	0.01	0.02	<0.01*
	Initial TBF (L)	1.56	0.99	2.13	<0.01*
TBF	Time (minutes)	0.08	0.02	0.14	<0.01*
	% TBSA	2.92	2.18	3.65	<0.01*
	Net fluid shift (ml)	0.02	0.01	0.03	<0.01*
	Initial TBF (L)	2.77	1.59	3.94	<0.01*

R_0 = resistance at zero frequency (ohms), R_i = intracellular resistance (ohms), R_{inf} = resistance at infinite frequency (ohms), ECF = extracellular fluid (L), ICF= intracellular fluid (L), TBF = total body fluid. *p= <0.05.

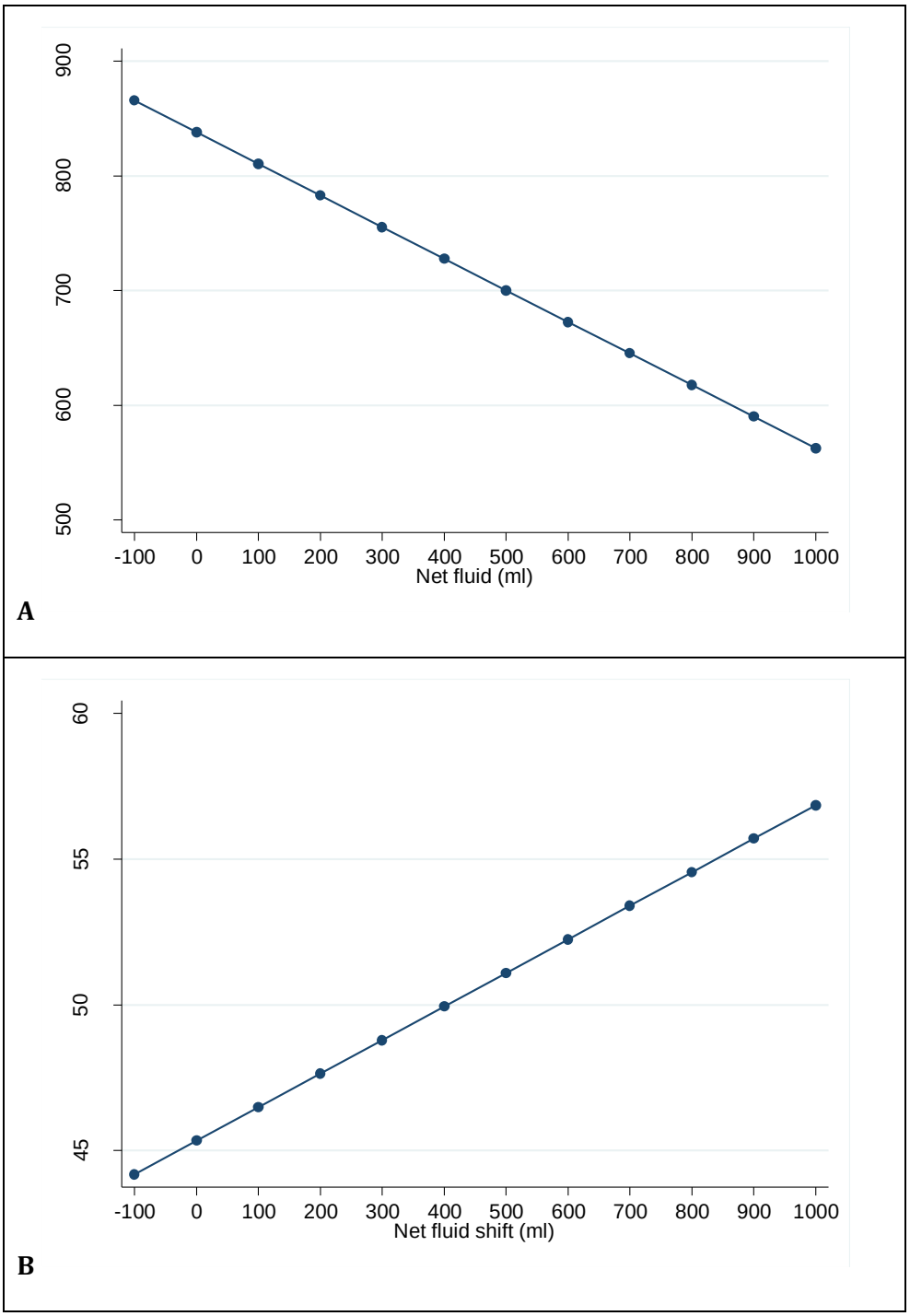

Figure 4.3: Predicted margin plots of BIS variable (Ri, ICF) and net fluid shift relationship

* The predicted margin plots of R_0 and R_{inf}, and ECF and TBF have a similar linear relationships to net fluid shift as R_i and ICF above. Ri = resistance of intracellular fluid, ICF = intracellular fluid

4.3.4 Calculator

A calculator was developed to estimate the net fluid shift between consecutive BIS measures, accounting for dressing condition, %TBSA and time since dressing (Appendix C). The significant and influential variables from the MLME models (Table 4.5) were incorporated into the newly developed algorithms (for calculation of fluid volumes), which were then embedded in an excel calculator to allow clinicians access to them. The variables required for input into the calculator by the clinician include dressing condition, %TBSA, time since application of Acticoat™ dressing (minutes) and the measured BIS variables. The calculator does not require the clinician to monitor or include net fluid shift, namely urine output and fluid input.

The validity analysis utilised the measured BIS fluid volumes and did not correct for the Acticoat™ effect, as it was not considered necessary for this preliminary study.

4.4 Discussion

The principal novel finding of this study show bioimpedance spectroscopy was a reliable method for monitoring fluid change in moderate to large burn patients. Bioimpedance resistance measures can be interpreted in the presence of Acticoat™ to monitor changes in fluid volume over time, if corrected for using the provided calculator. Thus, the study also established BIS as a valid indicator of fluid change over time during burns resuscitation while Acticoat™ dressings are in situ. Bioimpedance spectroscopy at the bedside has the potential to improve fluid management in an acute major burn by providing real time measures of fluid shifts thus reducing the risk of over resuscitation and associated adverse outcomes.

4.4.1 Reliability

The results of the study demonstrate BIS produces reliable raw and predicted measures in patients with >12% TBSA burns, regardless of dressing condition (open wound or Acticoat™) and electrode placement (Table 4.5). This data suggests BIS is a reliable method for assessing oedema change over time in moderate to large area burns. This concurs with and adds to the findings of Edgar et al's (2009) study which

found BIS reliability applicable to burns with <30% TBSA across different dressing conditions (28).

4.4.2 Factors influencing BIS readings

Bioimpedance whole body calculated fluid volumes were grossly and significantly overestimated and resistance of the ICF and TBF underestimated when an Acticoat™ dressing was in place. The under or overestimation of BIS variables increased with increasing TBSA. Grisbrook et al (2016) and Kenworthy et al (2017) also found the effect of silver dressings on BIS variable measures increased with increasing size of the burn.

Body mass index is also well known to be associated with BIS variable output as larger people have a greater amount of body fluid (38). This has been demonstrated in the present results where a larger initial TBF (indication of the bulk of the person and collinear with BMI) significantly decreased BIS resistance and therefore increased calculated fluid volumes.

It can be concluded that BIS was appropriate for use in a moderate to large burns population when an Acticoat™ dressing was in place only with adjustment, as resistance measures and fluid volumes are significantly under and overestimated with significantly different values to those in an open wound. The SFB7 impedimed embedded algorithms are not appropriate for use in burns with Acticoat™ insitu. This is consistent with the findings of Grisbrook et al (2016) (39) though the burns population sample in that study did differ from those recruited in this study sample with respect to %TBSA (range 5.5-28.5% compared to our 12-80%) and fluid resuscitation requirements. Therefore, to monitor fluid shifts it is recommended the resistance and fluid volume variables measured when an Acticoat™ dressing is insitu, be corrected using the provided calculator.

4.4.3 Validity

The present results show BIS is a valid indicator of fluid volume change over time in moderate to large burn resuscitation with TBSA, time, net fluid shift and initial TBF all significantly associated with BIS resistance and calculated fluid volumes. For

clinically interpretable resultsthe measured BIS variables need to be adjusted using the provided calculator if Acticoat^{TM} is in place.

Time was significantly associated with resistance variables, with an increase in time decreasing all estimated resistances and increasing ICF and TBF volumes. This may be explained by a combination of factors including the time since dressing application, the effect of Acticoat^{TM} and the amount of fluid resuscitation administered. Firstly, over time the Acticoat^{TM} dressing deposits more silver ions into the wound, therefore decreasing the raw resistance values and in turn increasing the 'equivalent' fluid volumes as calculated by BIS embedded algorithms (40). Secondly, the total mean volume of fluid resuscitation over time increased, thus increasing all inter-compartmental fluid volumes and consequentially decreasing the associated estimated resistance values. Although ECF was not associated with time, the p-value (0.15) is arguably low enough to accept that a clinical relationship may exist despite a small sample. In contrast, the embedded algorithm of analysis may explain why ECF is not associated with time in this population (each algorithm has different constants for estimating the individual fluid compartments (41)). However, R_0 the equivalent resistance of ECF significantly changed with time, suggesting fluid volume change in the extra cellular compartment is associated with time.

It is known BIS resistance is inversely proportional to fluid volume (22, 24). The results of this study support this. Bioimpedance variables and net fluid shift were found to have a negative inverse linear relationship with resistance and as expected, calculated fluid volumes a positive linear relationship (Figure 4.3) providing the net fluid shift (at each half hour measure) was greater than 100 ml. There were two patients who had a large (> 850 ml) negative fluid shift, both noted to have emesis during the single measurement period, and thus these data were excluded from the analysis, as they were assumed to have an altered, uncorrected physiological (ionic) state at the time of measurement and thus, significantly differed from others in the sample. It appears a large loss of fluid consequentially affects the following repeated BIS measures (within at least the following two hours). It is proposed that not only was the volume change a contributor to the difficulty in interpretation of the BIS measures but also the loss of electrolytes from the gut following emesis. The emesis could have altered the whole body fluid ionic state for a short period until it was

corrected by the body systems. Bioimpedance resistance is inversely proportional to fluid volume and electrolyte concentration. Therefore significant changes in the ionic status of the fluid or tissues measured will alter the BIS raw variables and render the machine embedded algorithms for calculated volumes, invalid. Clinicians are advised not to use BIS measures in the period after an episode of emesis (42). Further, the results suggest the BIS measure is only sensitive to fluid losses ≤ 100ml per half hour in the burns resuscitation period. The sensitivity of the BIS measure for fluid losses greater than 100 ml and less than 850 ml cannot be predicted as the patient cohort did not experience losses in this range.

4.4.4 Calculator

On the basis of the results a calculator was developed to improve the clinical utility of BIS in burns resuscitation patients at the bedside. It adjusts for the Acticoat^{TM} effect and provides an estimated change in BIS resistance and fluid volumes between consecutive BIS measurements, hence allowing fluids to be titrated accordingly. It has been established however that BIS is reliable and valid in the open wound condition. Therefore BIS can be utilised without variable adjustment when no dressings are in place.

4.4.5 Clinical Practice Recommendations

Optimum fluid resuscitation requires maintenance of the intracellular volume with minimal expansion (extravasation) of the extracellular volume. The results of this study indicate that using the relationship or pattern between R_0 or ECF and R_i or ICF is a non-invasive, interpretable method of monitoring or titrating fluid resuscitation. A stabilised R_i or ICF volume, over time, equal to or greater than the normal range (ICF 22.9-25 L) (24) represents a fluid resuscitation target. Fluid volumes should then be titrated to maintain R_0 or ECF at a steady state whilst continuing to preserve R_i or ICF at the target volume. Ideally ECF volumes would be maintained as close to normal (or the average for a healthy person) as possible (13.2-15.3 L). However due to the body's systemic "leaky vessel" inflammatory response to a major burn injury, with extravasation of fluid into the extracellular space, volumes within 5-10% of these norms would be a suggested acceptable target range (43, 44). In postoperative surgical patients fluid overload has been defined as >15% of preoperative fluid

volume (43) and in haemodialysis patients reaching ECF volumes within one to two litres of normal values is deemed acceptable (45). An example of how to titrate fluids: If R_i or ICF is stable and the change values of R_0 or ECF continue to increase, the fluid administered is adding to the extracellular compartment (swelling) rather than preferentially maintaining the intracellular compartment. Infused fluid volumes therefore need to be reduced if R_i (ICF) is stable and R_0 (ECF) is trending upward. However, in a recent study, intracellular volume actually decreased (~0.8L over 70 minutes) upon rapid infusion of intravenous fluid (~2L in ~ 60 minutes) into healthy male volunteers (46). It was suggested the infusion of fluid was responsible for the increase in extracellular fluid. The fluid administered in this study was <500ml/hr therefore difficult to conclude whether this may have the same effect. It however does suggest potentially accepting an ICF volume of ~ 1L less than average volumes when considering titrating fluid as above. For greater sensitivity to change, at this time this study suggests it is more advantageous to use the change in BIS raw resistance values (adjusted in the presence of Acticoat^{TM}) rather than the calculated volumes as it removes the need for specific predictive equations and eliminates the need for height and weight measures measures (47). There are a growing number of studies suggesting raw BIS variables may be more useful in predicting clinical outcomes (48, 49). BIS raw variables may also be able to indicate changes associated with cell membrane damage and cell wall integrity (49).

Further work is required to increase the confidence and promote greater utility of this sensitive measure over standard haemodynamic monitoring. In contrast urine output, a ‘quasi’ measure of fluid shifts and whole body perfusion (8) has been suggested to lag behind the actual events of hypoperfusion by up to two hours (50, 51). Bioimpedance also removes the need to rely heavily on initial fluid volume calculations such as the Parkland or Brooke’s. This could prove highly useful out in the field with paramedics and in isolated country hospitals where clinician’s burns experience may be limited and where Western Australia’s vastness means it is not uncommon for people to travel greater than eight hours to be admitted to a tertiary hospital.

4.4.6 Future Research

Additional research is warranted in evaluating the effect of other silver and non-silver dressings such as sulfadiazine and hydrocolloids, in moderate to large burns to increase the utility of BIS across burns services.

Further, consideration may need to be given of the type of resuscitation fluid (e.g. crystalloids versus colloids) in future studies as BIS electrical conductivity is affected by electrolyte concentration. This may therefore influence BIS variable measurements. Electrical and chemical burn injuries may also influence or change the ionic state of the tissue. Thus future research should include these modes of injury.

Ideally BIS would be able to be used on burns patients on life support or mechanical ventilation however further study needs to be done to determine whether electronic equipment interferes with the BIS instrument. Several studies have been conducted in intensive care units however they did not stipulate whether ventilated patients were included (52, 53).

4.5 Conclusion

In moderate to large burn patients, BIS is a reliable and valid method of oedema change. The Acticoat^{TM} dressings significantly alter the BIS raw outputs. To allow clinical interpretation of BIS, measures must be adjusted for silver dressings.

4.6 Acknowledgments

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Chapter 5 Bioimpedance Spectroscopy: A Technique To Monitor Interventions For Swelling In Minor Burns

This manuscript (study 3) was accepted for publication in the Journal of Burns, in April 2017.

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The manuscript was drafted and the submission completed by the PhD candidate, Pippa Kenworthy, accounting for ~85% of the intellectual property associated with the final manuscript. The remaining ~15% had contributions from the co-authors.

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Foreword

The first two studies presented in this thesis demonstrated BIS is a reliable method in the assessment of fluid shifts in acute burns >12%TBSA receiving fluid resuscitation, across different dressing conditions and electrode positions. Validity of BIS as a measure of fluid shift over time, with the use of the provided calculator to adjust for the presence of an Acticoat^{TM} dressing, was also established. Solutions to particular barriers in the use of BIS were also established. For broad clinical applicability across the spectrum of burns, BIS reliability and validity as a measure of oedema change needed to be determined in localised minor limb burns.

Minor burns experience localised wound oedema, not a systemic inflammatory response like major burns, and are also managed with both non-silver and silver dressings in the acute period. It is unknown whether whole body BIS is a sensitive measure of oedema change in minor burns, less than five percent TBSA. Therefore

standardised electrode placements and a novel localised electrode placement were investigated as well as the influence of dressing conditions on the BIS measures.

5.1 Introduction

Oedema as a result of inflammation is the body's normal response to injury (1). In burns, this process is exaggerated, causing an excessive volume of fluid in the tissues (2, 3). Oedema contributes to burn wound progression, slows healing and can increase risk of infection (4-7). Burn wound healing time is directly related to scar outcome (7, 8). Oedema can alter the severity of the wound by increasing the oxygen diffusion distance within the wound, exacerbating hypoperfusion thus forming a physical barrier to healing (9, 10). Limb oedema can also impact an individual's immediate physical function by limiting the range of motion of joints, causing pain with movement and mobilisation and increasing the effort required to move (11). Proactive, early management of oedema is therefore an integral part of a multidisciplinary intervention program to minimise the negative impact of swelling and optimise patient recovery (12). However, there is little high level evidence to support traditional oedema management regimes, nor is there emergent interventions for more proactive oedema removal (2). Thus, to guide improvements in oedema management in the burn population, a non-invasive, easy to use accurate assessment of swelling is required (13).

At present, the widely accepted methods for clinical monitoring of peripheral swelling are volume displacement and circumferential measures (14, 15). Circumferential measures are prone to subjective bias and lack sensitivity while volumetry is cumbersome to perform and rarely used in clinical practice (16). In the burns population both methods may pose an increased risk of infection, increased pain and can only be used when dressings are removed. Clinical examination of the burn wound such as visual analysis of depth, healing (re-epithelialisation) and signs of infection can also indicate presence of oedema (as a wound heals oedema decreases), however these are largely subjective (4, 17).

Techniques designed for the serial measurement of wound oedema would ideally be sensitive, reliable, user independent and minimally or non-invasive. Bioimpedance spectroscopy (BIS) is a technique, which may provide such a solution (18, 19). It is a

technique used frequently in healthy populations and more recently in clinical populations to measure an individual's body composition, including inter-compartmental fluid volumes, fat free mass (FFM) and cell (membrane) mass and function (20-22). By applying a small alternating current into the body via adhesive electrodes placed on intact skin, assessment of tissue resistance (R) and reactance (Xc) is possible. The R and Xc values are measured over a range of frequencies (5-1000hz). Bioimpedance spectroscopy software then utilises the Cole – Cole model applying non-linear curve fitting to estimate the resistance at zero frequency (R_0, extracellular fluid (ECF) equivalent), infinite frequency (R_{inf}, total body fluid (TBF) equivalent) (23). The intracellular fluid (ICF) resistance (R_i,) is extrapolated using the other raw variable data (24). Extra and intracellular fluids behave as resistive components and resistance is inversely proportional to fluid volume and therefore swelling (20, 25). The pathway the current takes is dependent on the frequency. At low frequency (5 Hz), currents travel through ECF only and at higher frequencies (>50 Hz) it travels through both ICF and ECF (26), thus providing the potential to develop a correlate measure of oedema volume (ECF).

Traditionally BIS technology measures fluid flux at a whole body level with electrodes placed in standardised locations on the hands and feet (23). However, segmental BIS, the measurement of the body in segments, brings the electrode-dependent field of measurement closer to the site of interest and is more sensitive to fluid volume changes of single limbs compared to whole body measures (25). Grimnes and Martinsen (2007) state as the distance between electrodes decreases, the deeper layers of tissue contribute less to the BIS result, therefore increasing sensitivity of the measured signal and oedema volumes (27). Codognotto et al (2008) used segmental electrode placement to assess single limb oedema as whole body electrode placement was shown to be insensitive to decreases in volumetric measures during the treatment of lymphedema (25, 28). Also in muscle injures, localised bioimpedance analysis (BIA) was able to detect changes in swelling and cellular injury consistent with MRI imaging over time (29). Localised BIA is not standardised and electrode placement differs depending on the site of injury or swelling. Electrodes are normally placed longitudinally and parallel to the axis of the limb. However, in a in a proof of concept study in uninjured adults, Ward et al (2013), demonstrated localised BIS to be a highly sensitive measure of fluid volumes

and highly reproducible data was obtained from electrodes located at different (localised) positions around the region of interest (19).

A challenge to measuring oedema in the acute burn environment with traditional methods is the presence of dressings and wounds. It is yet to be established if this invalidates, or is also a potential barrier to, the use of BIS. Acute burn wounds in the Western Australian context are dressed with a number of different products including hydrocolloid and ionic silver anti-microbials. Bioimpedance technology is based on the flow of an electrical current delivered at different frequencies through the body and is directly related to the amount of electrolyte rich (ionic) fluid in the field of measure. Therefore, silver (a conductive material) and hydrocolloid (water based) dressings have the potential to influence BIS variable outputs, independently of the oedema volumes in the tissues.

The ability to objectively assess local changes in fluid composition and fluid accumulation around the site of a wound would be helpful in determining the efficacy of the interventions currently aimed at reducing peripheral or limb oedema. Thus, the current study aims to examine the reliability and validity of the BIS technique for the measurement of localized burn wound oedema with respect to electrode position and dressing condition. It is hypothesised that bioimpedance resistance variables, R_0, R_i, R_{inf} will increase as limb volumes decrease.

5.2 Methods

5.2.1 Participants

A longitudinal, prospective, single service study was conducted between December 2014 and December 2016. Participants were included in the study if they were: over 18 years old, had a minor burn wound less than five percent TBSA, the injury was less than four days old and involved the limbs only and had a body mass index of between 15-40 kg/m^2. Patients were excluded if they were unable to lie supine for the duration of the testing. Manufacturer's requirements were adhered to thereby preventing inclusion of the following patients: pregnant or breast-feeding patients, patients with surgical implants and/or cardiac pacemakers.

Patients were initially recruited from Royal Perth Hospital (RPH) burns outpatient clinics or as inpatients on the RPH Burns Unit. Recruitment was then completed at Fiona Stanley Hospital (FSH) burns inpatient and outpatient areas due to the Western Australian State Burns Service moving in February 2015. The change of State Burns Service location did not alter the study protocol, patient population sampled or the equipment used.

5.2.2 Data Collection

Upon recruitment, participant's height, body mass, age and gender were recorded and input into the BIS device (SFB7 ImpediMed, Brisbane, Queensland, Australia). BIS measures were taken with participants in a supine position, limbs abducted away from the body and electrodes placed over cleaned, intact skin. The flow of the data collection process can be seen in Figure 5.1.

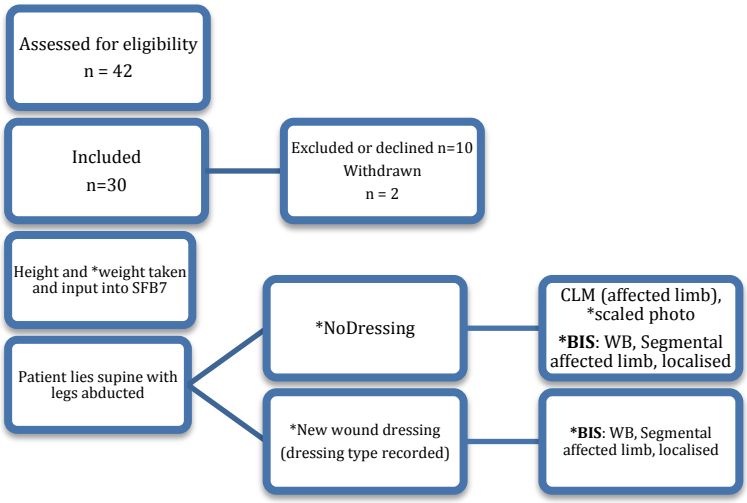


Figure 5.1: Flow diagram of data collection process. * ***Occurs at time point 1 and 2.***

5.2.2.1 Reliability

Circumference limb measures (CLM) of the 1) affected limb were measured to determine localised limb volume and 2) unaffected limb were measured to determine our raters' CLM reliability. Measurements were taken at three points. On initial assessment they were taken 3cm proximally and distally to the wound and at the mid point (across the wound) between these two measures. The proximal and distal measurement points were also measured in reference to specific anatomical

landmarks, with the patient supine in the anatomical position, so they could be replicated on the unaffected side and at follow up. This has been shown to increase the accuracy and reliability of circumference limb measures (30). The tape measure was cleaned with alcohol wipes to adhere to infection control protocols.

The localised sense electrodes were placed at the same measurement points as the proximal and distal CLM's, on initial assessment (Figure 5.2), making sure there was 3cm between the wound edge and the edge of the electrode. The distance between the two electrodes was also measured and recorded to 1) minimise electrode placement error at follow up and 2) to calculate localised limb segment volume. This inter electrode distance was termed the 'localised inter-electrode distance'.

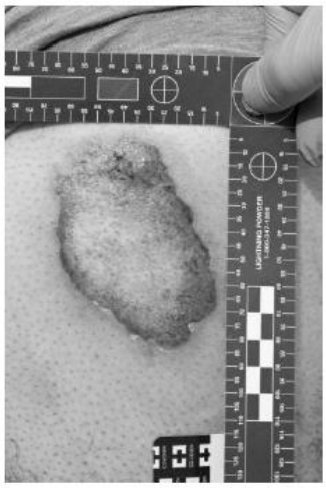
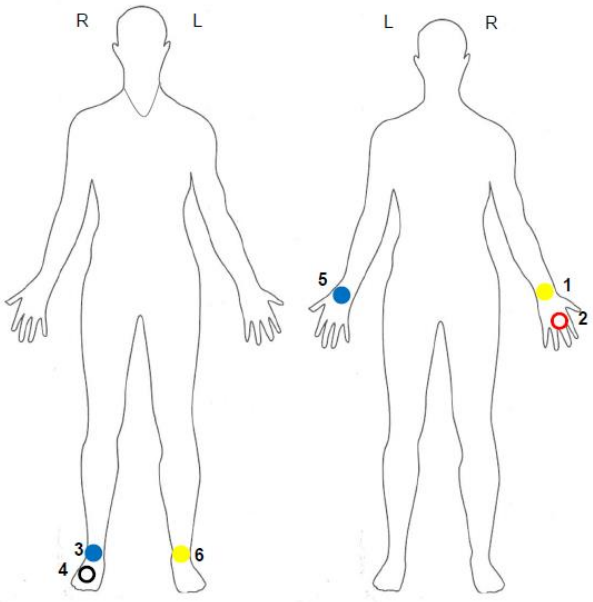


Figure 5.2: Position of distal localised electrode on a thigh, 3 cm from wound edge. Day 4 post burn.

Within each assessment session, triplicate BIS measures were taken for each electrode configuration (Figure 5.2 and Figure 5.3) and in both dressing conditions (no dressing and new dressing). Bioimpedance spectroscopy has been found to be reliable and valid in healthy and clinical populations (16, 31-33). In 2009, Edgar et al demonstrated the use of whole body BIS measurements of acute oedema shifts in human burn survivors, with injuries less than 30% total body surface area (TBSA) (mean 10.45% TBSA), with excellent reliability across different dressing conditions (16).



Electrode Montage	Electrode placement	
	Sense	Drive
Whole Body	1,3	2,4
Right Upper Limb	1,5	2,4
Right Lower Limb	3,6	2,4

Figure 5.3: Schematic representation of the BIS whole body and limb segment electrode positioning: measurement (sense) electrode sites (solid circles) and drive (current injecting) electrode sites (open circles).

5.2.2.2 Validity

BIS measures were taken in three different electrode positions to determine which is most sensitive to oedema change. They were 1) whole body BIS using standard tetra-polar whole body electrode placement (electrode position (EP_{WB}) (34); 2) whole limb segmental measures of the affected (EP_{limb}) limb (electrode placement as per Cornish, Jacobs et al 1999) (35) (Figure 5.3); and 3) localised BIS (EP_{local}) with the sense electrodes placed 3cm adjacent to the burn wound along the longitudinal axis of the limb (localised inter-electrode distance) and drive electrodes in the standard position (dorsum of the foot and hand) (Figure 5.3). This allowed a dressing to be accommodated where needed during the assessments. All measures were taken with 1) an open wound or no dressing and 2) with a new dressing (less than 2 minutes

old). The dressing type were categorised as either a non silver dressing or a silver dressing.

The volume of the novel limb segment was calculated using the truncated cone volume formula (36). Two truncated cone volumes were calculated for each localised limb segment and then added together (volume between mid and proximal, and mid and distal CLM).

$$V = [h (C_P^2 + C_P C_M + C_M^2)]/ 12 \pi + [h (C_D^2 + C_D C_M + C_M^2)]/ 12 \pi$$

Where:

$C_{P,M,D}$ = circumference limb measures (P = proximal, M = mid, D = distal)

h = height of each segment = localised inter-electrode distance /2

Participants had initial measures taken within 96 hours of injury (time point 1 (T1)) and follow up measures at a second time point (time point two (T2)), within fourteen days, after initial assessment to enable comparison of acute outcomes (BIS raw variables; localised limb segment volume) over time. It is known that in burns not requiring fluid resuscitation, oedema peaks on about day one post injury and by day four post- burn, the rate of volume change over time tapers to clinically insignificant levels (37). Therefore the method planned was to capture individuals within this initial time period to increase the likelihood of detecting changes in swelling over time.

Researchers (data analysers) were blinded to circumferential limb and BIS measures between time point one and two by using separate data collection sheets and only a BIS file name was recorded, not the actual variable values.

5.2.3 Equipment

The ImpediMed SFB7 was used to collect whole body, segmental and localised BIS measures. The equipment applies a small AC current across 256 discrete current frequencies (4-1000 Hz) to interpret each measurement. BIS computes both raw impedance values and derived fluid distribution values such as whole body ECF, ICF, and TBF using manufacturer's algorithms (23) and are stable when the subject's

body mass index is > 15 kg/m^2 (as per the manufacturer). Only raw BIS variables are used in this study, as the algorithm is not applicable to localised or segmental BIS.

Kendall CA610 diagnostic tab electrodes (reference code 31447793, Covidien, Mansfield, MA, USA) were utilised.

A thin 150cm tape measure was used for circumference limb measures.

5.2.4 Ethics

Approval for the study was granted by the RPH Human Research Ethics Committee (EC 2011/028), FSH governance committee (2014-106) and The University of Notre Dame Australia Human Research Ethics Committee (014139F).

5.2.5 Statistical Analysis

Stata Statistical Software, release 14 (StataCorp LP 2014, College Station, TX) was utilised to complete all data analysis. The Shapiro-Wilk test was used to assess the normality of the data. Normally distributed variables were described as means and standard deviations (SD). Where the data was not normally distributed, as for BIS measures, CLM and other co-variates, non-parametric statistics were performed. Where the variable was skewed it was transformed using the log function and the geometric mean and confidence intervals (CI) were reported, as for BIS resistances, localised inter-electrode distance, TBSA and CLM. A p value of less than 0.05 was considered statistically significant for all analysis.

5.2.5.1 Reliability

Two sample Wilcoxon rank sum (Mann-Whitney) tests were applied to determine if there were any significant differences between CLM of the unaffected limb between the two time points. As the patients were not undergoing resuscitation, it was assumed there would be no change in the size of the unaffected limb between sessions.

Reliability of the within session triplicate BIS resistance measures was determined by concordance (intra-class correlation coefficients (ICC)) (acceptable, 0.75-0.89; excellent, $\geq$ 0.9) (38), acceptable variance estimated by 95% confidence intervals

(95% CI) and systematic bias between trials (considered significant at $P < 0.05$) (38). The ICC's were obtained using a three level nested mixed-effects linear regression model. Multilevel mixed-effects (MLME) linear regression analysis was also utilised to determine if there was a significant change in BIS mean resistance values between triplicate measures. Initial and follow up triplicate BIS measures were included in the analysis.

5.2.5.2 Validity

A series of multilevel mixed effects (MLME) linear regression analyses were used to determine the effect of the measurement, patient and time characteristics on the dependent BIS variables (R_0, R_i, R_{inf}). Step-wise, backward elimination of the variables was completed, to produce the final model. A MLME linear regression analysis was also used to determine the effect of time on mean localised segment volume. The regression coefficients, with 95% confidence intervals were reported. A p value of less than 0.05 was considered statistically significant for all analysis.

The MLME model is a robust method allowing for nested observations of measures for each individual and provides a hierarchical analysis with generalisations for non-normalised data. The method can account for random effects from individuals and responses within individuals (39).

5.3 Results

5.3.1 Demographics

Thirty burn patients (20 males and 10 females) with a mean age of 37 (SD=10.57) years and a mean TBSA of 1.39% (SD=0.96) were included in the analysis. An additional two patients were excluded from the analysis. One was lost to follow up and the second due to equipment malfunction. The mean days post burn at initial recruitment (T1) was 2.35 days (SD 1.18,) and at follow up (T2) was 7.05 days (SD 3.98). The burns were located on upper limbs (n=16, 53%) and lower limbs (n=14, 47%) only. The localised inter-electrode distance mean was 18.19cm (CI 15.61-21.19). The total percentage of dressing by type in the final analysis was no dressing 53.23%, non-silver 30.07% and silver 16.70%. The median limb localised segment volume was 1861.94 ml (inter quartile range 850.63 ml– 3010.36 ml).

5.3.2 Reliability

The mean CLM scores of the unaffected limb at T1 and T2 for each point of measure are displayed in Table 5.1

Table 5.1: Geometric means of unaffected CLM (cm) at time point 1 and time point 2

Unaffected CLM point of measure	Mean CLM (cm) (CI) Time point 1	Mean CLM (cm) (CI) Time point 2
Proximal	33.19 (32.29-34.12)	33.52 (32.54-34.53)
Mid	29.27 (28.43-30.08)	29.66 (28.77-30.58)
Distal	23.98 (23.23-24.75)	24.69 (23.86-25.54)

CLM = circumference limb measures

A two sample Wilcoxon rank-sum test determined there was no significant difference between the medians (p value range 0.19-0.86) of the unaffected CLM (proximal, mid, distal) between repeated measures at the first and second time point. This indicates consistency of CLM's over time and between raters.

Table 5.2 presents the analysis of the within session triplicate BIS measurements reliability. There is a high correlation (level 3 intra class correlation) of the within session BIS triplicate resistance measures within the same electrode position, time point and dressing condition (BIS resistance is reliable in any circumstance) as determined by the ICC's which are as follows. R_0 0.9999 (CI (0.9999 - 0.9999); R_i 0.9999 (CI 0.9999 - 0.9999); R_{inf} 0.9999 (0.9999 - 0.9999). There were no significant differences between the estimated means of the within session triplicate measures for each of the BIS variables (p = 0.11-0.72).

Table 5.2: BIS reliability results

BIS Variable	Triplicate BIS within session measure	Co-efficient (CI)	p-value
R_0	2	-0.06 (-0.17 - 0.04)	0.257
	3	-0.02 (-0.12 - 0.09)	0.72
R_i	2	1.65 (-0.38 - 3.68)	0.110
	3	1.12 (-0.90 - 3.15)	0.278
R_{inf}	2	0.02 (-0.08 - 0.11)	0.721
	3	0.02 (-0.07 - 0.12)	0.602

*Triplicate BIS measures are in reference to the first triplicate measure

5.3.3 Validity

The series of MLME regression analysis conducted and univariate analyses established there was no significant effect of gender, age, weight, surgery, burn agent or burn depth on the BIS variables. Further associations and interactions between the independent variables and BIS resistance variables are reported below.

5.3.4 Effect of electrode position on BIS variables

Table 5.3 demonstrates the interaction between electrode position and time point. The BIS variables at localised EP (EP_{local}) had the biggest percentage change from T1 to T2 compared to the whole body (EP_{WB}) and affected limb (EP_{limb}) EP's as shown with the electrode position and time point interaction, however not significant. From T1 to T2 for EP_{local}: R_0 increased by 12% (p= 0.121); R_i increased by 12% (p=0.288); and R_{inf} by 11% (p= 0.241) whereas EP_{limb} had a percentage change less than 9% (p = 0.410-0.850) for all resistance variables, compared to EP_{WB}. Although none of the electrode positions and time point interactions was significant ($p \leq 0.05$), EP_{local} demonstrated the greatest power to detect change over time and was therefore the EP used for further MLME analysis.

Table 5.3: Change in BIS resistance variables with the interactions between time point and electrode position (in reference to time point 1 and EP1)

BIS Variable	Covariate Interactions	Co-efficient	95% Confidence Interval		*p*-value
			Lower	Upper	
R_0	Time point 2#Electrode position 2	1.02	0.87	1.18	0.85
	Time point 2#Electrode position 3	1.13	0.97	1.32	0.12
R_i	Time point 2#Electrode position 2	1.09	0.89	1.35	0.41
	Time point 2#Electrode position 3	1.13	0.90	1.39	0.29
R_{inf}	Time point 2#Electrode position 2	1.03	0.85	1.26	0.73
	Time point 2#Electrode position 3	1.11	0.92	1.36	0.24

= Interaction term. Time point#Electrode position interaction is in reference to time point 1 and electrode position 1 (whole body). Electrode position (EP) 2 = affected segment, Electrode position (EP) 3 = localised.

5.3.5 Effect and interactions of burn size, localised limb segment volume and dressings on BIS variables

Univariate analysis of time point determined R_0 and R_{inf} significantly decreased over time (p = 0.04 and 0.04 respectively). R_i did not change significantly over time (p = 0.07) (Table 5.4). When time, as indicated by assessment points (T1, T2) was included in the regression it did not significantly improve the prediction of any of the BIS variables using the MLME model. Time point was therefore not needed in the following analysis and results from the MLME regression analysis included EP_{local} from T 1 only.

Localised inter-electrode distance had a significant association with R_0 and R_{inf} only and TBSA had a significant association with R_0 only (p=0.05) (Table 5.4). As localised inter-electrode distance increased by 1cm, R_0 increased by 2.38 ohms (p= <0.01) and R_{inf} by 2.24 ohms (p=<0.01).

The mean volume of the burnt limb segment was significantly associated with each of the BIS resistance variables. A 1 ml increase in calculated volume reduced R_0 by 0.68 ohms (p = <0.01), R_i by 0.53 ohms (p = <0.01) and R_{inf} by 0.63 ohms (p= <0.01), indicating an inverse relationship between resistance and fluid volumes. Mean localised segment volume also changed significantly over time. From time point 1 to time point 2, mean volume had a mean decrease of 0.98 ml (CI 0.96-1.00) (p=0.05). There was no significant interaction between time point and mean localised segment volume for any of the resistance variables, suggesting the relationship between mean localised segment volume and resistance is consistent over time.

Regression analysis of the effect of dressing condition on BIS resistance values indicated there was a significant difference between no dressing and silver dressings at EP_{local} and T 1 for R_0 and R_{inf} measured. R_0 increases by 4.98 ohms (p = <0.01) and R_{inf} by 8.25 ohms (p = <0.01) with a silver dressing in place compared to no dressing (Table 5.4). A non-silver dressing also significantly increased resistance values in all measured BIS variables (p = <0.01) (Table 5.4) when compared to no dressing condition.

Table 5.4: BIS resistance measures relationships with covariates at electrode position 'local' only

BIS Variable	Covariate	Co-efficient	95% Confidence Interval		*p*-value
			Lower	Upper	
R_0 (ohms)	Time point 2	1.13	1.01	1.28	0.04*
	Localised inter-electrode distance (cm)	2.38	1.28	4.45	<0.01*
	TBSA (%)	1.43	1.00	2.16	0.17
	Non silver dressing	6.75	3.75	12.12	<0.01*
	Silver dressing	4.98	2.57	9.65	<0.01*
	Mean volume of localised limb segment	0.68	0.52	0.87	<0.01*
	Non silver dressing#mean volume	0.90	0.81	1.00	0.05*
	Silver dressing#mean volume	0.84	0.73	0.96	0.01*
	Non silver dressing# Localised inter-electrode distance	0.55	0.45	0.67	<0.01*
	Silver dressing# Localised inter-electrode distance	0.53	0.43	0.66	<0.01*
R_i (ohms)	Time point 2	1.17	0.99	1.40	0.07
	Localised inter-electrode distance (cm)	1.81	0.77	4.27	0.17
	TBSA (%)	1.42	0.84	2.41	0.19
	Non silver dressing	6.86	3.33	14.11	<0.01*
	Silver dressing	18.66	8.26	42.16	<0.01*
	Mean volume of localised limb segment	0.53	0.39	0.71	<0.01*
	Non silver dressing#mean volume	0.81	0.71	0.93	<0.01*
	Silver dressing#mean volume	0.81	0.68	0.97	0.02*
	Non silver dressing# Localised inter-electrode distance	0.55	0.43	0.71	<0.01*
	Silver dressing# Localised inter-electrode distance	0.32	0.24	0.42	<0.01*
R_{inf} (ohms)	Time point 2	1.17	1.01	1.36	0.04*
	Localised inter-electrode distance (cm)	2.24	4.14	16.44	<0.01*
	TBSA (%)	1.48	0.97	2.25	0.07
	Non silver dressing	6.78	3.68	12.48	<0.01*
	Silver dressing	8.25	4.14	16.44	<0.01*
	Mean volume of localised limb segment	0.63	0.48	0.81	<0.01*
	Non silver dressing#mean volume	0.88	0.79	0.99	0.03*
	Silver dressing#mean volume	0.81	0.70	0.94	<0.01*
	Non silver dressing# Localised inter-electrode distance	0.55	0.44	0.68	<0.01*
	Silver dressing# Localised inter-electrode distance	0.44	0.35	0.55	<0.01*

R_0 = resistance at zero frequency, R_i = intracellular resistance, R_{inf} = resistance at infinite frequency; # = interactions between 2 variables; *p= <0.05. Values for non silver and silver dressings are in reference to no dressing condition.

There was a significant interaction ($p < 0.01$-0.012) between silver dressings and the mean volume of the localised burnt limb segment for all resistance values (Figure 5.4). When a silver dressing was in place R_0, R_i and R_{inf} decreased with increasing volume, resulting in divergence away from no dressing R values as the limb segment volume increased (Figure 5.4 A,B,C). A significant interaction also existed between non-silver dressings and the mean volume of the localised burnt limb segment for all resistance values. When a non-silver dressing was in insitu R_0, R_i and R_{inf} decreased with increasing volume but resulted in a convergence toward the no dressing R value with increasing volume (Figure 5.4 A,B,C).

Significant interactions existed between the localised inter-electrode distance and each of the dressing conditions for all BIS R values at EP_{local} and T 1 (Table 5.4, Figure 5.5). A 1cm increase in the inter-electrode distance increased R_0 by 0.53 ohms ($P < 0.01$) when a silver dressing was insitu and the difference between silver dressing and no dressing R_0 increased as the inter-electrode distance increased (Figure 5.5 A). This relationship was opposite for R_i and R_{inf}, where R_i and R_{inf} decreased significantly ($p < 0.001$) with increasing inter-electrode distance with a silver dressing in place and resulted in divergence away from the no dressing R value with increasing inter-electrode distance (Figure 5.5 B,C).

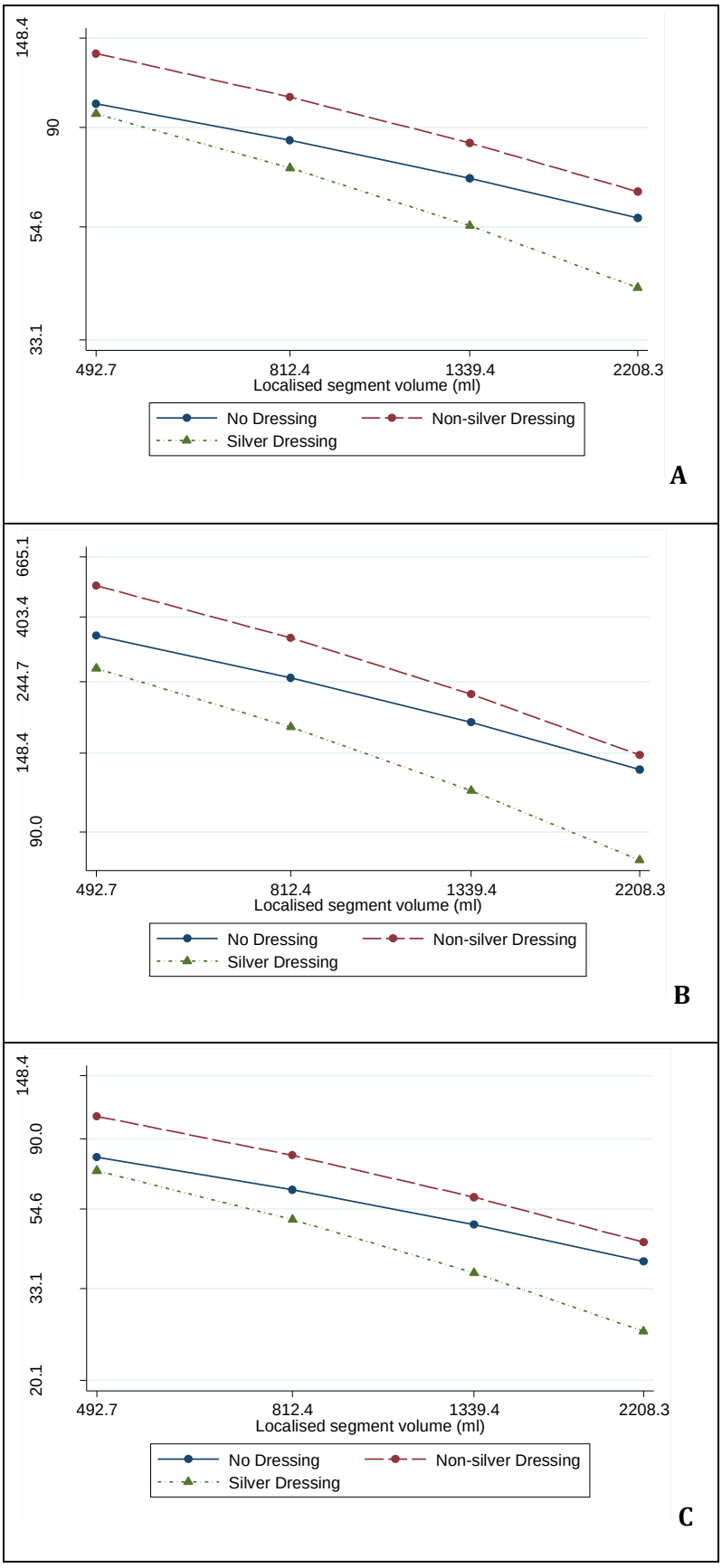


Figure 5.4: The interaction between dressing condition and mean localised segment volume (logarithmic scale) for R_0 (A), R_i (B) and R_{inf} (C).

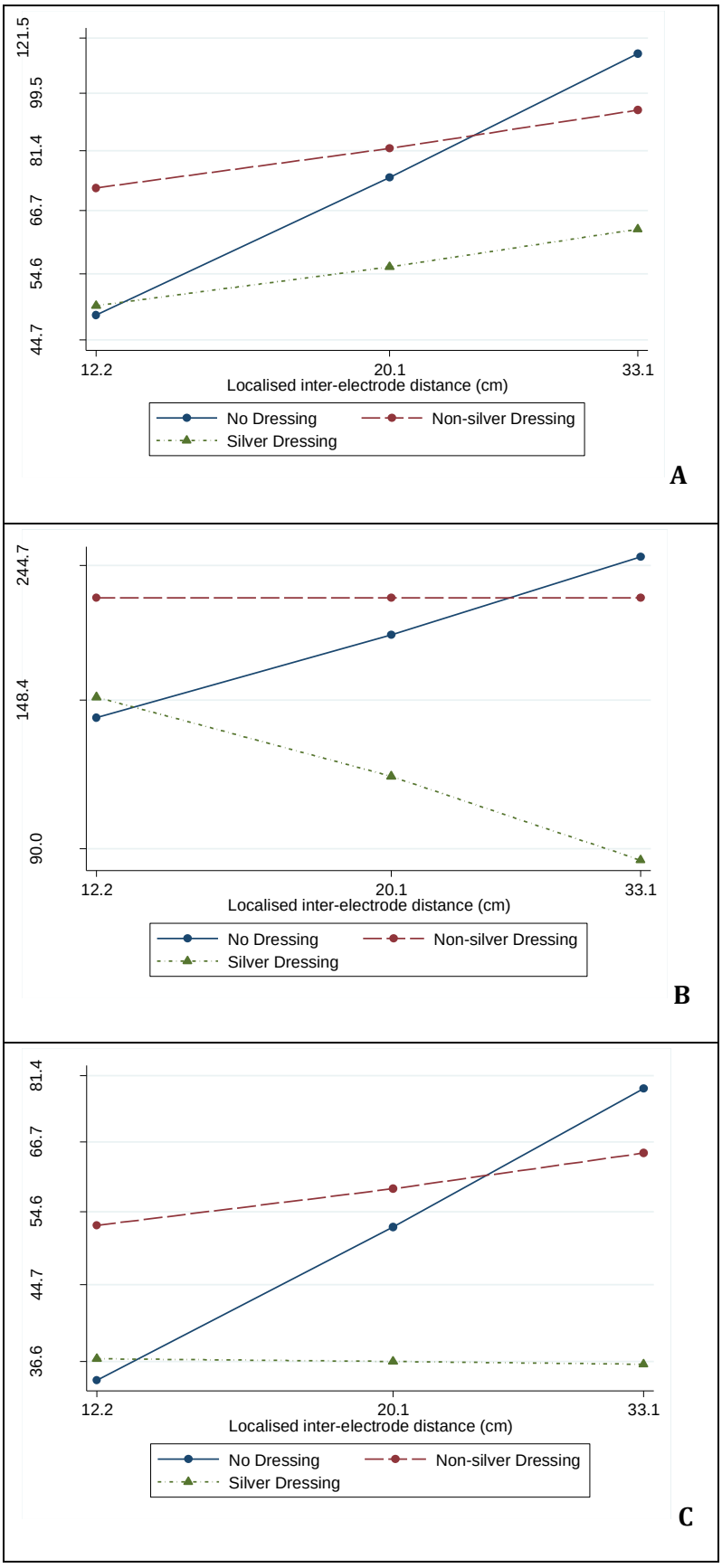


Figure 5.5: The interaction between dressing condition and localised inter-electrode distance (logarithmic scale) for R_0 (A), R_i (B) and R_{inf} (C).

There was a significant interaction ($p < 0.01$) between non-silver dressings and the inter-electrode distance for all resistance values (Figure 5.5). When a silver dressing was in place R_0, and R_{inf} increased with increasing inter-electrode distance, with the difference in R values between the no dressing and non-silver dressing conditions decreasing with increasing inter-electrode distance (Figure 5.5 A,C). R_i also had a significant and similar interaction but with minimal increase with increasing inter-electrode distance (Figure 5.5 B).

Algorithms were therefore developed to adjust R_0 when a dressing is in situ. They are as follows:

Adjusted (Ag) $R_0 = R_{0\ (Ag)}$ / (10.47 + 0.53* inter-electrode distance + 2.38* inter-electrode distance)

Adjusted (non Ag) $R_0 = R_{0\ (Non\text{-}Ag)}$ /(12.24 + 0.55* inter-electrode distance + 2.38* inter-electrode distance)

Where:

$R_{0\ (Ag)}$ = measured BIS R_0 when a silver dressing is in place

$R_{0\ (Non\text{-}Ag)}$ = measured BIS R_0 when a non-silver dressing is in place

Inter-electrode distance = the measured inter-electrode distance

The above equations can be used to adjust R_0 when BIS is used in the presence of any dressings, thus providing a measure of oedema change.

5.4 Discussion

In patients with minor limb burns, resistance as measured by BIS is a reliable and valid index of oedema change. In patients with an acute wound, this study demonstrated that localised BIS was sensitive and accurate for use with and without a dressing in situ. Further, to improve the clinical application in burns, the interpretation of the BIS resistance variables is enhanced by adjusting for the presence of a silver impregnated and non-silver dressings. From the results of this study, adjustment of BIS resistance is now possible due to the development of an

algorithm, which can be embedded as formulae in readily available spread sheet software.

The SFB7 instrument provided reliable BIS resistance output regardless of dressing condition and type, and electrode position. The data demonstrated high intra-class coefficients (>0.999) with minimal variance (95% confidence interval range 0.996-0.999) and no indication of significant systematic bias. This suggests BIS is a reliable tool for monitoring changes in BIS R values in minor limb burns. These results are consistent with the literature (40). Edgar et al (2009) found BIS to be a reliable method for assessment of oedema shifts in burns (%TBSA < 30%) regardless of dressing condition and dressing age (16). Circumferential limb measures of the unaffected limb were also found to be reliable with no significant difference in measures found over time. This indicates the CLM's and thus truncated cone volume estimates of the affected limb in this study were reliable, as the unaffected limb volume was not expected to change appreciably between time points. This is due to minor burns causing a localised inflammatory response (not systemic) with swelling concentrated around the burn wound (2, 3, 41).

A primary aim of this study was to establish whether BIS is a valid measure of oedema volume change in minor limb burns (<5% TBSA) with respect to electrode position and dressing condition. A localised (EP_{local}) electrode montage was found to be the most sensitive arrangement when compared to the calculated truncated cone measurement and when compared to whole body and segmental electrode positions. Localised BIS electrode placement was best option to detect and measure oedema volume change over time. The change in resistance values detected over time ranged from 0.11- 0.12% at electrode position 3 and 0.2 - 0.9% at electrode positions 1 and 2. This compares similarly with other studies where localised electrode positions were superior at detecting change in fluid volumes (42, 43). It has been demonstrated that narrowing the field of measurement closer to the site of interest increases the sensitivity of bioimpedance measures (44). As localised electrode positioning is not standardised to manufacturer's specifications, it is recommended strict measurement and placement protocols be adhered to, to ensure consistency (comparability) of BIS assessments and minimisation of the introduction of type two measurement error, on the same individual.

The MLME regression analysis allowed us to accept our hypothesis, bioimpedance resistance variables, R_0, R_i, R_{inf} increase as limb volume decreases (Figure 4 A,B,C). Additionally, all BIS resistance values, R_0 and R_{inf}, at EP_{local} had significantly increasing mean values (1.13-1.17 ohms) over time and the burnt limb segment volume significantly reduced over time. It is known BIS resistance is inversely proportional to fluid volumes and therefore swelling (33, 45). Burn wound healing clinically manifests as reduced oedema, in the acute phase (3, 46). This suggests BIS resistance variables can monitor changes in minor acute burn wound oedema over time and is supported by Ward et al (2006) (47). They reported raw resistance values can be used as a surrogate index of volume due to the inverse relationship between the two. Further, there was a greater percentage change in BIS resistance variables (R_0 5.27%, R_i 7.68%, R_{inf} 8.80%) over time than with burnt limb segment volume (0.13%), indicating BIS is more sensitive to fluid volume change than calculated truncated cone volume measures from CLM. This concurs with Cornish et al 2001 who found BIA was 100% sensitive at detecting those at risk of lymphoedema and CLM had a sensitivity of only 5% (43). The study demonstrated that BIS has a superior ability to detect small oedema volume changes in a clinical setting, compared to CLM, and thus could be better placed to help guide early decisions and oedema management practices.

After establishing the reliability and validity of BIS in patients with a wound, the focus of this study following on was to examine the influence of silver impregnated and non-silver dressings in assessment of limb oedema in the clinical context. Dressings certainly render other common assessment techniques such as WDV and CLM, uninterpretable. In this study we found regularly used silver impregnated burns dressings significantly affected BIS resistant values. As expected, due to the delivery of ionic silver from dressings, BIS resistance values decreased compared to the no dressing conditions, measured in the same session. In addition, the difference increased as the 1) localised inter-electrode distance increased and 2) limb segment volume increased. This is consistent with the findings of Grisbrook et al (2016), who documented that silver dressings interacted similarly with TBSA in burns with a median TBSA of 15%, where an increasing %TBSA had a measurable decrease in BIS resistance variables when a silver dressing was in situ (48). As the localised inter-electrode distance increases, the greater the depth of the BIS current and

therefore the greater amount of tissue it passes through. This explains the significant relationship of increasing R_0 and R_{inf} values with increasing localised inter-electrode distance (44, 49).

Non-silver dressings also significantly affected within session BIS resistance variables compared to no dressing conditions. However the measured resistance values were increased in comparison to no dressing conditions and the difference decreased with increasing 1) localised inter-electrode distance and 2) limb segment volume. Hydrocolloid dressings, the main non-silver dressings used in this patient sample, contain gelatin and cellulose and are adhesive (50). Gelatin is a highly viscous protein and coupled with the adhesive properties may act like a cell membrane or skin, thus resulting in a measured increase in BIS resistance (51).

To increase the clinical utility of BIS at the bedside the provided algorithm can provide adjusted R values when a dressing is in situ. Resistance at zero frequency, equivalent to ECW and therefore oedema, would be the most clinically useful BIS variable to track changes in oedema volume. The localised inter-electrode distance, significantly associated with BIS R values, is a measure that can be taken clinically with or without a dressing insitu and with relative ease and accuracy, unlike CLM. In minor burns it can be used as quasi measure of percent TBSA, as TBSA estimation can be highly variable and inaccurate (52). Localised inter-electrode distance was therefore included in the final MLME regression analysis, instead of limb segment volume, to estimate BIS R values and formulate the algorithm to adjust the BIS R variables when a dressing is insitu.

It is recommended localised BIS be utilised to improve responsiveness of the BIS measures. As long as the localised inter-electrode distance and the measured BIS R (with dressings) is entered into the provided algorithm BIS can be used in a clinical setting to assess oedema change over time and the effectiveness of treatment interventions.

Bioimpedance spectroscopy was demonstrated to be more sensitive in the assessment of oedema volume change, than traditional methods. It is simple and rapid to use. Anecdotally, during this study it took ~two minutes to complete measures from set up to finish, This concurs with other authors who have quoted one minute for BIS

measures versus seven minutes for tape measurements in the assessment of limb lymphoedema (53). They also reported BIS is better accepted by clinicians, therapists, and patients than serial CLM or WDV methods. It is non-invasive and can be used accurately with dressings in place or an open wound (54). Further, it is more sensitive to oedema volume changes over time than truncated volume measures. Therefore, the BIS methodology can provide the earliest possible objective data regarding oedema volume and guide management interventions in the same timeframe. Thus, BIS has the potential to assist in limiting the impact of adverse outcomes associated with burn wound oedema.

5.4.1 Future Studies

This study examined the use of BIS in minor limb burns and the effect of electrode position and new dressings on the measured resistance variables. To enhance clinical utility however, investigation of the effect of dressing age is necessary as the properties of common dressings change with time. Silver dressings deposit silver ions over a particular amount of time and hydrocolloid dressings absorb fluid and wound exudate forming a gel. Both conditions are likely to affect the electrical conductivity based on the principles that resistance is proportional to the amount of electrolyte rich fluid (23). Clinically this is relevant as minor burns often have dressings left in place for up to five days with physical rehabilitation and oedema management strategies occurring within this time period, thus necessitating oedema monitoring in these timeframes. Further studies may also include quantifying limb and whole body oedema with BIS, so a true magnitude of oedema change over time can be measured. The magnitude of change in research is relevant to determine the best intervention however further research is required to determine this. This was beyond the scope of this study. In addition, investigation of BIS in the assessment of oedema shifts in major burns and the effect of regularly used silver dressings on BIS variables would further enhance the clinical applicability of BIS in the burns population.

5.5 Conclusion

Localised bioimpedance spectroscopy is a reliable, valid and non invasive technique for the assessment of oedema after minor limb burns with and without dressings in

situ. BIS provides an interpretable measure of oedema change in minor limb burns when dressing condition is accounted for using the provided algorithm.

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Chapter 6 Monitoring Wound Healing In Minor Burns – A Novel Approach

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Foreword

The first 3 studies have demonstrated the reliability of BIS across different electrode positions and dressing conditions and have established the validity of BIS, after adjusting for dressing condition, as a measure of fluid change across the spectrum of burn severity. As a result of these previous studies, it is possible to recommend BIS as an adjunctive objective measure of oedema and fluid shifts in burn injured patients, major and minor, which will i) assist in improving clinical assessment and treatment of oedema and ii) aid oedema management intervention studies aimed at reducing the overall impact of burn severity.

The final study of this thesis explores BIS, as an objective measure of monitoring burn wound healing. Localised BIS is able to monitor wound healing in traumatic and surgical wounds and has been shown to be more sensitive at detecting wound infections than regular laboratory tests. It is not known however, if BIS can monitor wound healing in acute minor burns.

6.1 Introduction

Wound healing, re-epithelisation, is a complex but well described physiological process. Erythema, heat, pain and swelling are classic symptoms of both acute and chronic wounds, which are caused by a vascular and cellular inflammatory response of the body to injury (1). Timely treatment of the wound and associated symptoms is crucial for providing the best possible environment for healing. Acute burn wounds are unique in their degree of swelling. After a burn the body responds with an influx of chemical and inflammatory mediators resulting in excess swelling (2).

Immediate management of a burn wound should include optimum first aid, management of swelling and medical attention with appropriate dressings (3, 4). Improvements in dressings, surgical intervention and the advent of antibiotics over the decades has improved aspects of burn wound care, yet oedema remains an issue. It is known that in the first 3-5 days post burn when assessed according to Jackson's three zones of tissue injury (5), the burn wound can progress, thus increasing the wound depth and time to healing and increasing the risk of a worse scar and functional outcome (6). Time to healing is directly related to severity of scarring (2).

Oedema is considered a primary impediment to the healing process and burn wound conversion (7). The specific mechanism by which oedema interferes with healing is unknown but is theorised to be related to compromised vascular and tissue diffusion dynamics (8). Peri-wound oedema is thought to impair the clearance of cellular debris and waste; to prevent the migration of inflammatory cells impairing defence from infection and antigens; and impeding nutrient transport from the capillary bed to the cell (9). Other factors affecting healing are an individual's pre-morbid health and age. Systemic factors such as diabetes, peripheral vascular disease and obesity are associated with slowed wound healing (10).

It is essential to monitor wound healing closely to ensure the most appropriate intervention to promote healing is carried out. In clinical practice the assessment of a burn wound must include the wound size (total body surface area (TBSA)), depth, agent and days post burn. Each of these factors helps guide the best and optimal medical management of the patient (11). Other signs such as wound oedema volume

and chemical changes in the wound surface are essential assessment points and may indicate infection (1).

The most common measures used to assess a burn wound are: visual evaluation; photos, TBSA and depth (determined by colour, skin elasticity, hairs left) (12, 13). These are influenced by a certain degree of subjectivity and clinician specialisation and training. Clinician assessment of a burn wound has been shown to be accurate only 60–75% of the time (14). The use of computer software, planimetry, wound biopsy, laser Doppler and ultrasound can be used to objectively assess the structure of the wound but these can be expensive, require specialist training and don't necessarily provide immediate results (15). In the burns wound environment, dressings may remain in place for 2-5 days, limiting wound assessment unless dressings are removed. Having the capability to monitor wound healing with a dressing in place would limit dressing cost, decrease patient burden and pain and potentially detect infection and delayed or poor healing in wounds earlier. Kenworthy et al (2017) (the authors of this study) found bioimpedance raw resistance measures, can monitor localised changes in acute burn oedema with dressings in place (16). In addition, the ability to monitor wound healing in real time, non-invasively and without subjectivity would be advantageous and minimise error.

Bioimpedance spectroscopy (BIS) is an instrument with this potential. It can measure the body's inter-compartmental fluid volumes, indicate metabolic state and cell health through passing a small electrical current, over a number of frequencies, via electrodes placed on the skin and measuring the voltage drop between them (17). The current flows depending on the body's composition. The body offers two types of resistance to an electrical current. They are resistive R (resistance) and capacitive R (reactance) (18). Resistance is the opposition to flow of an electric current and capacitance is the delay in the passage of current through the cell membranes and tissue interfaces. The BIS instrument measures real time raw variables (resistance (R), reactance (Xc) and phase angle (PA)) using current frequencies of 4-1000 kHz. Mathematical formulas embedded in the BIS instrument then utilise these raw variables to estimate the inter-compartmental fluid volumes (19).

Resistance has an inverse relationship to fluid volume due to alterations in electrolyte concentration, so as the fluid volume increases R decreases. Resistance at zero (R_0)

frequency theoretically indicates extracellular fluid ((ECF) oedema), as the current does not traverse the cell membrane. Higher frequency currents pass through the cell membrane and ionic extracellular environment, therefore R at infinity frequency (R_{inf}) indicates total body fluid (TBF) (20). Practical limitations prevent the use of zero frequency (direct currents) and low high frequency alternating currents, therefore values of R_0 and R_{inf} are predicted by the BIS instrument using a Cole-Cole plot (21). Resistance of the intracellular fluid (ICF) (R_i) is extrapolated using the other raw variable data. Reactance represents cell membrane mass and function. Phase angle, calculated as the arc tangent of Xc/R and expressed in degrees (18). The capacitance of the cell membrane causes the current to lag behind the voltage as it traverses the cell, creating a phase shift of the waveform as measured by BIS (22). If the health of the tissue is disturbed in any way (e.g. inflammation, disease) the electrical properties of those tissues (cell membranes) are altered. Phase angle is therefore promoted as a measure of cell membrane health and a prognostic indicator of malnutrition and disease (23). As the health of the cell improves, the transit of the BIS current and voltage is delayed, thus resulting in greater PA's. In experimental case studies, BIS R and PA measures have been, shown to be associated with wound healing in acute and chronic wounds (24-26). Resistance measures were also positively associated with histological measures of healing in surgically induced wounds in rats (26). The following study therefore aims to examine whether the BIS technique is a valid measure of wound healing.. Based on the evidence from the literature it is hypothesised R and PA will increase with burn wound healing.

6.2 Methods

6.2.1 Participants

Participants were recruited from the Western Australian State Burns Service, outpatient clinic between December 2014 and December 2016.

Participants, who were over 18 years of age, had a minor limb burn (less than five percent TBSA) which was less than four days old were eligible for inclusion in the study. Participants were able to be included if they also had minor burns to other non-assessed body locations and, or if they had surgical intervention to the burn

wound of interest. They were excluded if they had a body mass index (BMI) < 15 kg/m2 and were unable to lie supine. Manufacturer's contradictions also excluded pregnant or breast-feeding individuals, participants with surgical implants and cardiac pacemakers.

This was a longitudinal study, with participants having BIS, circumference limb measures (CLM) and photos taken on two different days. Patients were initially recruited within four days of injury and followed up in a second measurement session within 14 days of initial assessment.

6.2.2 Data Collection

6.2.2.1 Wound Healing

Digital photos (in colour), were standardised by inclusion of a measurement scale in each image, and were taken of the individual's burn at initial recruitment and follow up to visually monitor wound healing area. A Burns Attending Surgeon reviewed the scaled photographs and determined whether there was healing of the burn wound over time. Indicators such as epithelialisation (assessed by wound hue and wound surface moisture), presence of erythema and wound area were used to assess the wound. A combination of these factors were utilised to categorise the wound. The wound healing categories and relevant descriptors are as follows: 1) worse – increased area, worsening erythema, wound hue changes indicating burn wound conversion or increased wound ooze, signs of infection; 2) no change – no clear difference in the wound, in any stated parameter as per category 1, could be seen on visual assessment and 3) healing – re- epithelialisation, decreased wound area, wound contraction, increasing red/pink hue of the wound. The parameters of wound healing were assessed at follow up to provide a category of wound healing in comparison to the initial assessment.

6.2.2.2 Bioimpedance Spectroscopy

The subject's height, body mass, age and gender were recorded and entered into the BIS instrument (SFB7 ImpediMed, Brisbane, Queensland, Australia). Participants were positioned in supine with limbs abducted away from the body. Electrodes were placed over cleaned, intact skin with the measurement (sense) electrodes placed 3cm longitudinally either side of the burn wound (Figure 1). Drive (current applying) electrodes were placed in the standardised position, at the head of the third metacarpal dorsally and the base of the third metatarsophalangeal joint dorsally. To minimise inter- and intra-rater error, bony anatomical landmarks were used as measurement reference points for placement of the two sense electrodes, with the patient in supine and the distance between the sense electrodes was also measured (27). Within each assessment session localised BIS (R_0, R_i, R_{inf} and PA) measures were taken in triplicate with an open wound. Phase angle measured at 50 kHz (PA50) was utilised as it has been suggested to be the most appropriate frequency to monitor changes in bioimpedance variables in humans (28).

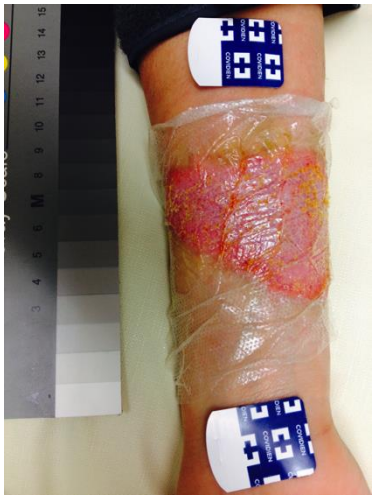

Figure 6.1: Burn of volar forearm two days after surgery. Sense electrodes in place either side of wound

6.2.2.3 Localised limb segment volume (oedema)

The localised limb segment volume was calculated using the truncated cone method, as a method of oedema assessment. In minor burns, oedema peaks on day one (1) post injury and then reduces to clinically insignificant levels by day four (4). Wound healing clinically manifests itself as reduced oedema (29). Therefore limb segment

oedema volume was determined to support and strengthen the statistical analysis and primary aim .Limb circumference measures were taken at the site of the sense electrodes (distal edge) and at their mid point with the patient in the anatomical position. These CLM were then utilised to calculate limb segment volume using the truncated cone method (27). Reliability of our CLM has been determined in a previous study (16). The tape measure was cleaned with medi-wipes to adhere to infection control protocols.

Truncated volume measures of the localised segment were determined using the below formula.

$V = [h (CP2 + CPCM + CM2)]/ 12 \pi + [h (CD2 + CDCM + CM2)]/ 12 \pi$

Where:

C P,M,D = circumference limb measures (P = proximal, M = mid, D = distal)

h = height of each segment = inter electrode distance /2

Researchers were blinded to the CLM and BIS measures between recruitment and follow up. The Burns Attending Surgeon was also blinded to both the BIS and CLM results.

6.2.3 Equipment

Localised BIS measures were collected using the ImpediMed SFB7 instrument (ImpediMed, Brisbane, Queensland, Australia) (Figure 6.2).

The portable BIS instrument applies a small AC current across 256 discrete current frequencies (4-1000 kHz) via electrodes placed on intact skin. Electrical leads connect the electrodes (via alligator clips) and the BIS instrument together. Patient's details are entered via a touch screen. BIS measures raw R and Xc values and then computes PA (at the varying BIS frequencies) as the arc tangent of Xc/R.

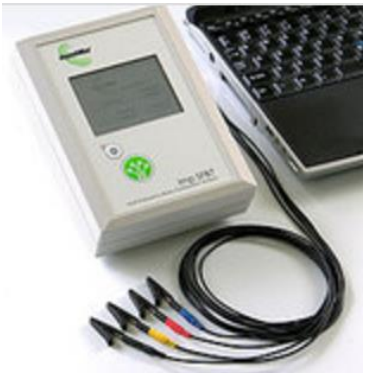

Figure 6.2: ImpediMed SFB7 instrument (ImpediMed, Brisbane, Queensland, Australia)

Kendall CA610 diagnostic tab electrodes (reference code 31447793, Covidien, Mansfield, MA, USA) were utilised.

A tape measure was used for circumference limb measures (CLM) to calculate truncated limb volume and Fiona Stanley Hospital (FSH) medical illustrations department photographed the wounds with a standardised technique.

6.2.4 Ethics

Approval for the study was granted by the Royal Perth Hospital (RPH) Human Research Ethics Committee (EC 2011/028), and subsequently Fiona Stanley Hospital (FSH) Governance Committee (2014-106) (upon transfer of the Burn Service to the new hospital during the study period) and The University of Notre Dame Australia Human Research Ethics Committee (014139F).

6.2.5 Data Analysis

Statistical analysis of the results was completed using Stata statistical software, release 14 (StataCorp LP 2014, College Station, TX). Normality of the data was assessed using skewness – kurtosis tests. Descriptive statistics (mean ± standard deviation) were utilised to portray normally distributed patient characteristics and appropriate predictor variables.

Non-parametric statistics were performed where the data was not normally distributed. A Spearman's rank-order correlation was performed to determine the relationship between a healing wound and the mean limb segmental volume. The

results are presented as the correlation co-efficient (rho) (weak, 0-0.39; moderate, 0.40-0.59; strong, > 0.6) (30). Kruskal-Wallis equality of populations test was applied to determine if limb segment volume was different for the three groups of wound healing (worse, no change, healing). The results of the Kruskal-Wallis test were reported as χ^2. A p value of less than 0.05 was considered statistically significant for all analysis.

A series of proportional-odds ordered logistic regression (POLR) analyses, were used to determine the effect of BIS variables R_0, R_i, R_{inf}, PA50 and limb segmental volume on the dependent categorical variable, wound healing. Wound healing as confirmed by epithelialisation and area. The odds ratios, with 95% confidence intervals were reported. Statistical significance was determined if the p value was less than 0.05. Prior to interpreting the results of the OLR models; 1) several assumptions were evaluated, confirming the response variable healing is ordinal and, healing is linearly related to each BIS variable and 2) Step-wise, backward elimination of the variables was completed, to produce the final model.

6.3 Results

A total of 30 patients with minor limbs burns <5% TBSA were recruited and a final 28 (20 male, 10 female) were included in the analysis. Two patients were excluded, one due to equipment malfunction and one lost to follow up (did not return for second assessment). Patient injury details are presented in Table 6.1.

Table 6.1: Patient injury details (n=28). Values presented as means and (standard deviations) or number, where appropriate.

TBSA of Ax wound	Days post burn		Burn Location		Wound Healing Categories			Surgery
	Initial Ax	Follow up Ax	Upper limb	Lower limb	Worse	No Change	Better	Yes
1.39% (0.96)	2.35 (1.18)	7.05 (3.98)	16	14	5	2	21	6

Ax = assessed

Within this patient sample, burn wound depths included superficial partial thickness (n=9), mid dermal (n= 11), deep partial thickness (n=6) and full thickness (n=2). The

surgical intervention included dermabrasion and ReCell® (Visiomed group ltd) (n=3) and split skin graft and ReCell® (n=3). The median limb localised segment volume was 1861.94 ml (inter quartile range (IQR) 850.63 ml – 3010.36 ml). The median limb localised segment volumes by wound category were 'worse' 3010.48 ml (IQR 1587.64 – 3231.84 ml), 'no change' 1221.15 ml (IQR 1081.52 – 1360.78 ml) and 'healing' 969.57 ml (IQR 509.86 – 1810.44 ml).

Spearman's correlation determined there was a significant but weak negative association between a healing wound and limb segment volume (ml), rho -0.30, p <0.01.

Kruskal-Wallis tests determined that there was a statistically significant difference in limb segment volume between the three wound healing groups, $\chi^2 = 9.62$, $p = 0.008$. However, the non-healing wound response category sample sizes were small (worse, n = 5; no change, n = 2) and the results should be interpreted with caution. An analysis of variables with sample size less than five (5) per category cannot be considered a robust result (31).

Proportional-odds ordered logistic regression analysis determined surgery was a significant predictor variable of healing. Once surgery was adjusted for, R_0 and R_{inf} were significantly associated with healing. A one ohm increase in R_0 and R_{inf} will increase the odds of wound healing by 6% and 5% respectively (Table 6.2). Phase angle and R_i were not significantly associated with healing of the wound. Whilst limb segment volume was correlated with wound healing (spearman's analysis), when added to the POLR analysis it was not significantly associated with the wound healing categories i.e.it did not enhance prediction of wound healing outcome compared to BIS variables, and thus was not warranted in the final POLR model. Burn wound depth was not significantly associated with wound healing category (p = 0.85).

Table 6.2: Relationship of wound healing with localised BIS variables

Wound	Covariate	Odds Ratio	95% Confidence Interval		p-value
			Lower	Upper	
Healed	R_0 (ohms)	1.05	1.02	1.08	<0.01*
	R_i (ohms)	1.01	1.00	1.03	0.07
	R_{inf} (ohms)	1.06	1.02	1.11	<0.01*
	PA50 (degrees)	0.94	0.67	1.32	0.74

R_0 = resistance at zero frequency, R_i = intracellular resistance, R_{inf} = resistance at infinite frequency; PA50 = phase at 50Hz; *p= <0.05. BIS = bioimpedance spectroscopy

6.4 Discussion

In patients with minor limb burns, localised BIS resistance measures, at zero and infinite frequencies, are able to monitor wound healing. BIS demonstrated a significant association with a healing wound and the subsequent decrease in oedema volume which supports this result.

The POLR analysis allowed us to confirm part of the hypothesis that bioimpedance resistance variables (R_0, R_i, R_{inf}) will increase as the wound heals. The results determined R_0 and R_{inf} significantly increased with wound healing, but Ri and PA did not. These results are supported by rodent and human studies where epithelialisation of a wound was associated with an increase in resistance measured at a variety of frequencies (24, 26, 32). Lukaski and Moore (2012) suggest R is a specific biomarker of cell growth where increases reflect healing and decreases is suggestive of a lack of healing.

Resistance at zero frequency increasing with wound healing may also be explained by the reduction in limb oedema with healing. At low frequencies the BIS current cannot penetrate the cell membrane and is therefore a measure of ECF. In the acute phase, one element of burn wound healing is a reduction in oedema (7, 33). This is further supported by the significant negative spearman's correlation between a healing wound and limb segment volume (ml), and the difference in limb volumes in each of the three wound healing categories (worse, no change and healing) as determined by Kruskal-Wallis analysis. The spearman's correlation and Kruskal-Wallis analysis results also indicate that reduction of edema volume is a measureable

symptom of progression of acute wound healing. The Kruskal-Wallis analysis result though, needs to be interpreted as a preliminary finding due to the low sample numbers in two of the wound healing categories. Segmental limb volume however, was not a significant independent predictor of wound healing in the multivariable POLR analysis. Measurement of resistance at high frequencies, R_{inf}, is reflective of molecules inside and outside the cells (TBF). Resistance at infinite frequency would therefore increase as a result of decreased oedema and cell proliferation (34). Changes in R_i are not reflective of wound healing and may be due to the intracellular fluid compartment remaining stable in an acute minor burn wound.

In this study, the PA measured by BIS at a frequency of 50 kHz was not significantly associated with healing of a minor limb burn for this cohort. Therefore, using the markers of wound healing defined for this study, there is no evidence in this sample that PA50 measurements are related to wound healing. Phase angle indicates the distribution of water between intracellular and extracellular space and reflects the electrical integrity of vital cell membranes (35). Wagner et al (1996) found localised PA measures taken at two different sites were significantly different between those at high risk of pressure ulcers compared to a control group. There was however no difference in PA within the high risk or control groups between the two sites (36). It is therefore possible, in minor limb burn injuries, that the relationship between Xc and R is consistent independent of the extent of tissue injury. In contrast however, localised PA50 has been demonstrated in a series of case studies using serial measures of wounds with varying aetiologies, to reflect wound healing and breakdown (23). An alternative explanation for the inconclusive findings regarding phase angle as an indicator of wound healing from this analysis may be due to the lack of sensitivity of the wound healing markers used in this study i.e. visual assessment and, or the limitation of a small sample size in the wound healing categories, worse (n = 5) and no change (n = 2). A second explanation of the PA results measured at 50 kHz may not be the optimum frequency that is sensitive enough to measure cellular proliferation in acute burn wounds. Tornuev et al (2014) demonstrated that a PA at higher frequencies (100 or 200 kHz) is best to distinguish a change in the level of cellular healing and thus is more sensitive in detecting wound healing and inflammatory diseases in mammary glands after surgery (37). In contrast, Kekonen et al (2012) found healing of a single superficial acute wound

could be first detected with BIS at frequencies between 1 - 100 kHz within the first four days of injury. Frequencies lower than 1 kHz did not indicate any significant change in the wound. It appears optimal PA frequency for measuring healing may differ for different wound aetiologies.

This study demonstrates the capability of BIS as a quantitative non-invasive index of wound healing. The BIS measures are sensitive which allows some confidence in the generalisation of our results. A limitation of the study however is the small overall sample size, which is not a representative sample of the burns population and does not allow conclusive results to be drawn with respect to PA as a wound healing measure.

To further enhance the clinical applicability of BIS in burns, studies investigating the association of PA at various frequencies with burn wound healing using enhanced wound healing markers and larger sample sizes are warranted. In addition assessment of bioimpedance until wound healing would provide a greater understanding of the relationship between BIS variables and the wound healing process.

6.5 Conclusion

Bioimpedance spectroscopy resistance variables, R_0 and R_{inf}, can be used to monitor wound healing in minor limb burns as an adjunct to standard practice. Further research is required however to investigate if phase angle is of value as an indicator of the wound healing process.

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Chapter 7 Synthesis Of Results And Conclusions

7.1 Introduction

The oedema that occurs after an acute burn has a significant negative impact on wound healing and in severe cases, patient survival (1). The rate of wound healing is directly related to the severity of the scar, which consequentially can significantly affect the functional and psychological well-being of the patient (2-4). There is therefore an urgency to reduce acute burn oedema. Whilst considerable gains have been made in many areas of burn care, few advances have been made in the treatment and measurement of acute burn oedema in both minor and major burns. Advancements in acute burn oedema management have been stunted by the ability to measure the efficacy of interventions (5, 6).

Following a burn injury, significant oedema is present in measurable amounts for up to 5 days, as fluid leaks (fluid shift) into the tissue from the inflamed blood vessels (6). The management of this fluid shift in major burns involves formal fluid resuscitation. Adjustment of the patient's fluid requirements is a dynamic process and close monitoring is recommended, in order to prevent under or over resuscitation in the first 24 - 48 hours after burn the burn injury. When treating a burn this way, the clinician treads a fine line between excess tissue oedema, which slows wound healing and increases the risk of scar; and the prevention of hypovolaemic shock, renal failure and possibly death. The current most widely utilised measures of oedema and fluid shift include CLM, urine output monitoring and WDV. However they either lack precision, are invasive and/or lack utility in the acute burn unit. Urine output is a 'quasi' measure of fluid shifts and whole body perfusion and is suggested to lag behind actual hypoperfusion events (7). These limitations and the challenges of oedema volume change assessment in burns was the driving force behind this series of studies. A rapid, real time, reliable method of oedema assessment is required to help reduce the negative sequelae of acute burn oedema.

Bioimpedance spectroscopy, is emergent in the literature as a method to evaluate oedema change in burns. It has advantages of other competing technologies of oedema change assessment such as near-infrared spectroscopy (NIR), perometry and

ultrasound as it has demonstrated reliability, sensitivity in detecting fluid volume change, is practical and user friendly (1, 8, 9). Also, after initial purchase BIS is low cost and likely a sustainable method of oedema monitoring in comparison to other technologies.

Bioimpedance spectroscopy is a non-invasive tool, which is based on the principle that the resistance to the flow of an electric current through the body is directly related to the composition of the body. By measuring the resistance of the whole body and the limbs it is possible to calculate the inter-compartmental fluid volumes of the body (and other tissues) and hence obtain an index measure of oedema. The utility of BIS, as a non-invasive measure of fluid shifts in burn patients has been previously demonstrated, however the studies lacked power to determine BIS as a valid measure of fluid shift (1, 10, 11). The use of the BIS method in acute burns is also hindered by the presence of open wounds at the sites of standard electrode placement, i.e. the hands and the feet and by the presence of dressings. The dressings routinely used in the first 24-48 hours of injury, in the BSWA, incorporate silver compounds and dressings after this period are commonly hydrocolloid or similar. Since bioimpedance is based on the flow of an electrical current through the body, it raises the question as to whether the accuracy of the BIS measures is altered in the presence of various dressings.

This research therefore aimed to investigate an alternative method, which is easy and rapid to use, for monitoring fluid shifts in the acute burn environment. Hence, the primary aim was establishing whether BIS was a reliable and valid instrument for measuring fluid shifts in acute burns, across the spectrum of burn severity. Secondly, to address the potential barriers to the clinical application of the BIS instrument in this environment and thirdly to examine whether BIS could monitor minor burn wound healing.

The outlined research problems were addressed using four integrated studies. This chapter: 1) summarises the outcomes of each of the studies, 2) discusses the clinical limitations of BIS, 3) considers the future path of research and 4) concludes with the significance, recommendations and clinical implications of the research.

7.1.1 Study 1: Addressing The Barriers To Bioimpedance Spectroscopy In Major Burns: Alternate Electrode Placements

The first study in the study series addressed potential barriers to the use of BIS in burns receiving fluid resuscitation to enable greater clinical utility by investigating alternate electrode placements when wounds hinder the use of standardised placement. The literature reports movement of electrodes circumferentially around the limb, theoretically, will not affect BIS measures (12). However Grisbrook et al (2015) found BIS measures were significantly different when electrodes were moved circumferentially on the lower limb in healthy populations. In contrast, movement of electrodes proximally 1cm and 2 cm has been reported to result in a change of mean BIS resistance values by 2% and 4% respectively (13). The use of alternate electrode placements in the burns patient population has not been described in the literature. The single service study therefore specifically aimed to contribute to the body of knowledge and determine whether alternate electrode configurations for whole body and limb segmental BIS outputs were comparable to standardised electrode configurations in moderate to large size burns across different dressing conditions (an open wound and Acticoat^{TM} dressing).

The first study demonstrated that whole body bioimpedance spectroscopy resistance variables (R_0, R_i, R_{inf} indicative of extracellular, intracellular and total body fluid respectively) and extracellular fluid (ECF) volumes were interchangeable in an open wound and the Acticoat^{TM} dressing condition. All upper limb segmental measures were interchangeable in an open wound only but not in an Acticoat^{TM} dressing. The differences between measurements of other BIS variables (namely intracellular fluid (ICF) and total body fluid (TBF) whole body measures and all lower limb measures) across the dressing conditions were not clinically acceptable. It was also evident that the Acticoat^{TM} dressing condition amplified the differences between the standard and alternate electrode positions but also between the open wound and Acticoat^{TM} dressing condition for each BIS variable. The study however was not designed to explore this effect further. Additionally, it was shown that the standardised whole body BIS fluid volumes measured in the open wound environment were comparable to those expected when fluid resuscitation volumes were taken into consideration

(14) i.e. the ECF, ICF and TBF volumes were within 4 L (maximum resuscitation volumes) of normal values. This further supports the establishment of BIS as a valid measure of fluid volume change in the burns environment. Also, the fact that resistance variables were more stable than fluid volumes between the two electrode configurations (standard and alternate) may suggest that resistance measures are more clinically useful as it removes the need for predictive equations (15).

The results of the study therefore demonstrates whole body alternate electrode placements are a feasible alternative when wounds preclude the use of standardised placement for monitoring R_0, R_i, R_{inf} and ECF within dressing conditions in burns >12% TBSA. This result hence partly ameliorates the potential difficulties to the use of BIS in the burn population, improving its practical application in this clinical environment. Further research is required to establish the best alternate electrode placements to measure all BIS variables in moderate to large burns and to therefore enhance its clinical utility.

7.1.2 Study 2: An Objective Measure For The Assessment And Management Of Fluid Shifts In Acute Major Burns

The second study, in the study series, expands on addressing the barriers to BIS application in burns and explores its reliability and validity as a measure of fluid shift (using both raw resistance variables and calculated fluid volumes). The reliability and applicability of BIS in the measurement of fluid volumes in the burns environment has been demonstrated yet it has not been validated as a method of fluid shift assessment (10, 12). The second observational longitudinal study therefore aimed to contribute to the understanding of a) the reliability of BIS with respect to dressing condition and electrode position, b) the influence of Acticoat^{TM} on BIS variable outputs and c) the validity of whole body BIS to assess net fluid shift in the presence of moderate to major burns.

This study demonstrated the reliability of BIS under any dressing conditions and electrode position. All BIS measures were reliable within any electrode position (standard and alternate whole body and limb segmental), across dressing conditions (open wound and Acticoat^{TM}) and over time. We therefore propose that

bioimpedance spectroscopy is a reliable method for monitoring fluid change in moderate to large burns patients.

Further, this study supported the hypothesis that "Acticoat dressing used in the first 48 hours of burn injury in the BSWA reduced BIS variable outputs". Acticoat^{TM}, an antimicrobial silver impregnated dressing significantly reduced BIS resistance variables as expected, and led to increased calculated fluid volumes. There was also a significant Acticoat^{TM} TBSA interaction where the Acticoat^{TM} effect on BIS measures was magnified with increasing TBSA. These results concur with those found by Grisbrook et al (2015). Therefore, in order to maximise clinical utility of BIS in the measurement of oedema at the bedside, this PhD project has included the production of algorithms embedded in a calculator to adjust for the effect of Acticoat^{TM} on BIS fluid volume measures.

The final hypothesis that 'BIS raw resistance variables will decrease and predicted fluid volumes will increase with increasing fluid shift' was also accepted. Bioimpedance variables and net fluid shift were found to have a negative inverse linear relationship for resistance and calculated fluid volumes a positive linear relationship providing the net fluid shift, between consecutive measures, was greater than -100 ml. Other factors influencing BIS measures were: initial TBF volumes, with increasing initial TBF increasing measured fluid volumes and decreasing measured resistance and; time, where increasing time decreased resistance variables and increased fluid volumes measured.

This study confirmed that BIS is a reliable and valid indicator of fluid volume change in moderate to large burns, if BIS measures are corrected for using the provided calculator and the fluid shift is not larger than -100 ml i.e. the patient can't have a loss of fluid >100ml within the consecutive time periods. The calculator is able to adjust for the effect of Acticoat^{TM} and can provide an estimated change in BIS fluid volumes between consecutive BIS measurements (e.g. half hourly or hourly intervals), hence providing the potential for fluids to be titrated accordingly. This finding is important as BIS provides immediate, non-invasive assessment of fluid volume, thus having the potential to reduce the risk of over or under resuscitation and associated adverse outcomes. Other methods of fluid shift monitoring in the acute burn resuscitation period involve invasive monitoring or

delayed results from laboratories (16, 17). And the most widely used outcome measure, urine output has been suggested to lag behind the actual events of hypoperfusion by up to two hours questioning its accuracy (18, 19). Further work is required though, to increase confidence and allow greater reliance on this sensitive measure in fluid resuscitation management, over standard haemodynamic monitoring. Figure 7.1 shows a summary flow chart for the use of BIS in major burns.

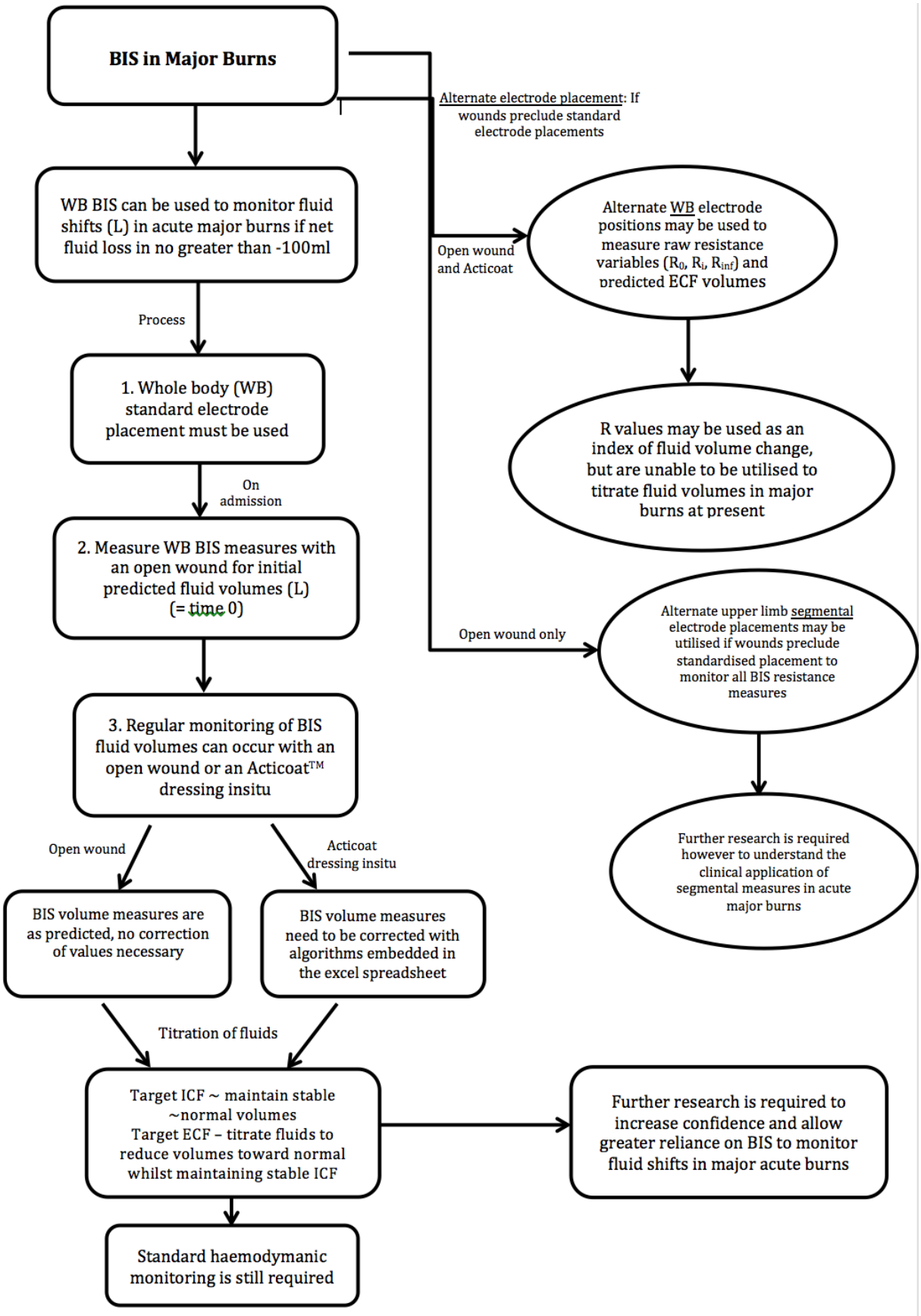

Figure 7.1: Summary flow chart for the use of BIS in major acute burns

7.1.3 Study 3: Bioimpedance Spectroscopy: A Technique To Monitor Interventions For Swelling In Minor Burns

The first two studies in the series demonstrated BIS is a reliable and valid measure of whole body fluid volume change if measures are corrected for the presence of dressings by using the using the developed calculator. These studies attempted to address barriers to the use of BIS in the acute burns resuscitation environment by investigating alternative electrode placements and the effect of routinely used dressings in the BSWA. The third study continues in the same vein, exploring BIS as a reliable and valid measure of oedema change in minor burns with respect to dressing condition and electrode placement. Limb segmental and localised BIS has been shown to be a reliable and sensitive measure of lymphoedema and oedema change in muscle injuries (9, 20) but whole body BIS has not (21). Yet, it is unknown if this is true in minor burns less than five percent TBSA. Therefore, the third study examined the reliability and validity of the BIS technique for the measurement of localised burn wound oedema with respect to electrode position and dressing condition.

This study supported the hypothesis that BIS variables R_0 (resistance of extracellular fluid), R_i (resistance of intracellular fluid), R_{inf} (resistance of total body fluid) increased as limb volume decreased. This finding is supported by Ward et al (2006) who reported raw resistance values could be used as a surrogate index of volume due to the inverse relationship between the two. It was also determined that localised BIS was the most sensitive electrode positioning to detect oedema change (R_0), was reliable and the BIS raw resistance measures provided a valid index of oedema change in minor burns. Additionally, BIS was found to be more sensitive to fluid volume change than calculated truncated cone volume measures from CLM.

As with the major burn study (second study) dressings were found to influence the BIS measures and they had a significant interaction with TBSA. It was found hydrocolloid dressings increased and silver impregnated dressings decreased the measured BIS resistance. An algorithm was therefore developed to adjust resistance values when dressings are in use. This improves the clinical utility of BIS to monitor

localised changes in burn wound oedema. These findings expand the usefulness of BIS in the burns population. There is now a rapid, reliable and valid objective measure of peripheral oedema that can be utilised in the presence of dressings and wounds. Unlike CLM and WDV, the most widely accepted methods of peripheral oedema assessment, where their use is limited to open wounds, and they pose an infection risk if cleaning procedures are not thorough. Bioimpedance spectroscopy's utility in the monitoring of peripheral oedema change is valuable as being able to determine the effectiveness of oedema management interventions easily, can guide best patient care and help improve functional and scar outcomes post burn.

7.1.4 Study 4: Monitoring Wound Healing In Minor Burns – A Novel Approach

The final study explores BIS as a method of monitoring wound healing. As with assessing oedema changes, usual assessment of wound healing involves an undressed wound. The most common current assessment tools are computer software packages, which assess the area and depth of wound, and subjective assessment by specialised clinicians (22). Bioimpedance spectroscopy is emerging as a tool for wound healing assessment both in rodent and human studies (23, 24). It has been investigated in chronic non-healing wounds of differing aetiologies (e.g. traumatic, surgical) and in surgical mammary gland wounds (25, 26). All BIS variables appear to be associated with healing, however the totality of studies performed in this area seem to primarily investigate resistance and phase angle as indicators of wound status.

The fourth and final study determined the BIS technique is a valid measure of wound healing and examined whether a healing wound is associated with oedema volume change.

The hypothesis that 'BIS resistance increased with burn wound healing' was partly confirmed. It was determined that the resistance of the extracellular and total body fluid (R_0 and R_{inf} respectively) were associated with a healing wound, each increasing as the burn wound heals. An increase in both of these resistance values is related to a reduction in oedema with a healing wound. This was further supported by the significant negative correlation between a healing wound and limb segment volume (ml). Kekonen et al (2015) found resistance, measured at varying

frequencies, increased with epithelialisation of an acute wound (27). The results however found phase angle (PA and promoted as a measure of cell membrane health) at 50 kHz was not significantly associated with healing of a minor burn for this cohort. Therefore, using the markers of wound healing as per this study, there is no evidence in this sample that measurements of PA at 50 kHz are related to wound healing. Further research is warranted to explore the capability of BIS as a non-invasive tool for quantitative evaluation of wound health with PA's at different frequencies. It has been demonstrated that BIS frequencies of 100-200 Hz are more sensitive to early changes in indicating wound healing and is worth investigation in burn patients (26).

It can be concluded that BIS resistance values at zero (indicative of oedema) and infinite frequencies can be used, in conjunction with standard practice, to monitor the status of minor burn wounds. Figure 7.2 provides a summary of how localised BIS is utilised in minor burns.

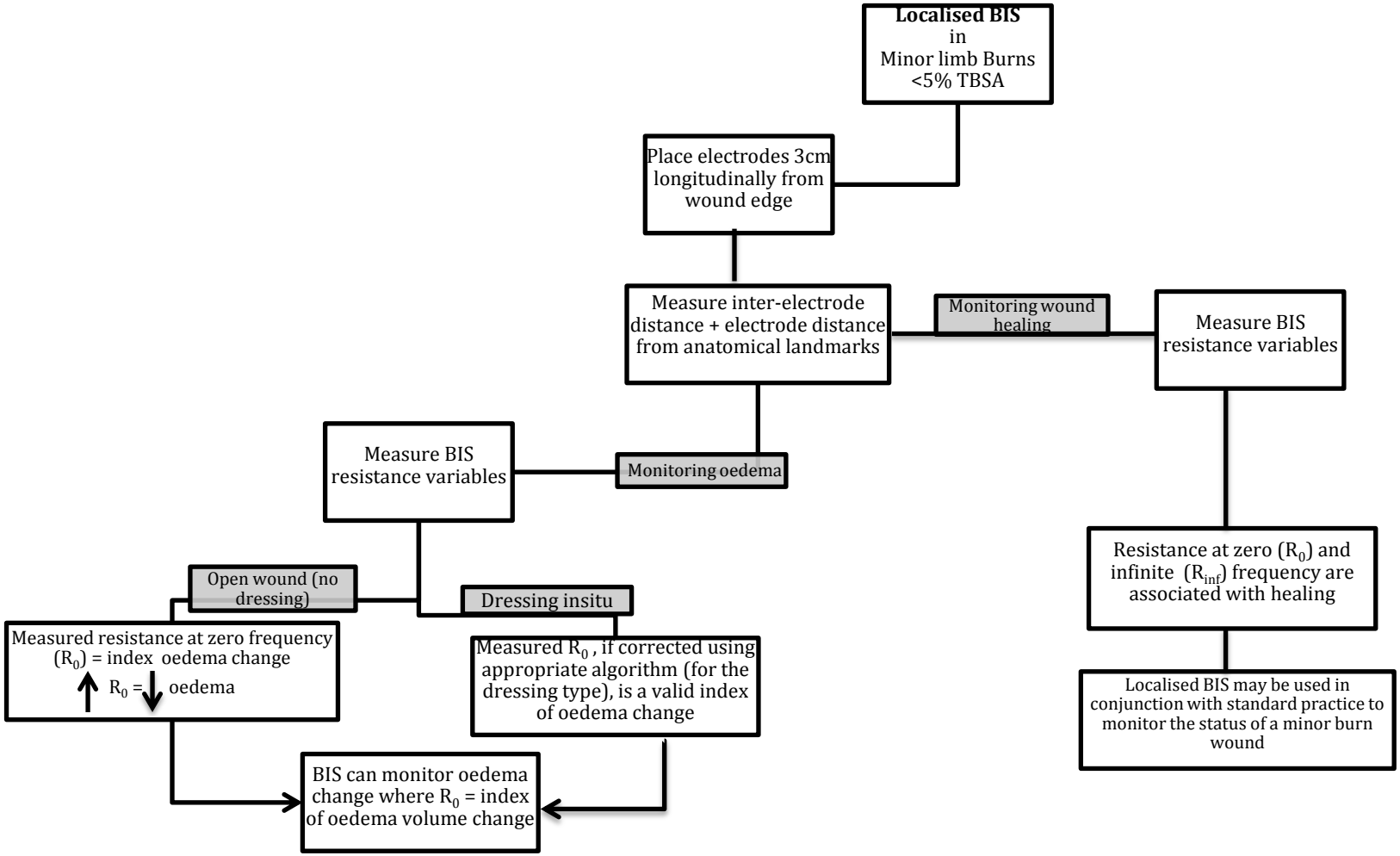

Figure 7.2: Summary flow chart for the use of BIS in minor acute burns

7.2 Limitations

Even though the individual studies presented in Chapters three to six discuss their limitations, these were guided by the journal requirements. Further limitations relevant to the individual studies are detailed below.

7.2.1 General Limitations

This study was limited in terms of the population involved. It was a single service study including adults only and results may not able applicable to paediatrics. This is due to the different developmental stages of children and varying body composition throughout these stages, which significantly influence BIS measures (28). Furthermore, being a single service study allowed only dressings routinely used in the BSWA within the specified timeframes to be investigated. This may limit the generalizability of the results to other services, which use different dressings, especially silver impregnated ones, in the acute period. The population was also limited to acute burns, potentially decreasing the application and generalisation of results to subacute and chronic burn oedematous states.

7.2.2 Study 1: Addressing The Barriers To Bioimpedance Spectroscopy In Major Burns: Alternate Electrode Placements

A limitation of this study was the use of the same alternate lower limb electrode positions utilised in the healthy population by Grisbrook et al (2015). Due to the time constraints of the research and schedule of the researchers we were unable to await the results of their study. We had to move forward with the proposed alternate electrode placements, based on the theory of equi-potentials from the literature (12). Grisbrook et al (2015) demonstrated the lower limb alternate electrode placements did not provide interchangeable BIS measures with the standardised positions. Knowing this, we could have investigated other alternate electrode placements but were unable to. This is therefore considered in the future research. The standard and alternate electrode positions were only measured in a new Acticoat^{TM} dressing condition so results may not be generalizable to measures in older dressings.

Acticoat™ works by depositing silver ions into the wound over time thus likely increasing the conductivity of the BIS electrical current, in turn affecting BIS measures.

7.2.3 Study 2: An Objective Measure For The Assessment And Management Of Fluid Shifts In Acute Major Burns

The results from this study are not generalizable to major burns with dressings other than an open wound or Acticoat™. Study Three demonstrated that non-silver impregnated dressings alter BIS measures. Due to other research projects being conducted at the BSWA site, time constraints were put on the research and the project was limited to collection of data within the first 48 hours of injury only. Hence other dressing conditions were unable to be included in this study, as Acticoat™ is the dressing used in this timeframe in the BSWA. Investigating the relationship between BIS measures and total body weight changes (a gross measure of oedema change) was also limited. Burn dressings and retention of fluid from formal resuscitation in large burns pose a barrier to regular reliable weights in the acute period.

7.2.4 Study 3: Bioimpedance Spectroscopy: A Technique To Monitor Interventions For Swelling In Minor Burns

Confirmation of localised BIS as a measure of localised wound oedema ideally should have been compared to WDV rather than CLM, however in collusion with the supervisors of this candidature it was not considered viable during this project. Firstly, to be able to include limb burns at any location (upper or lower limb) large containers of water would be required which are cumbersome, heavy and pose a risk to the researcher. Secondly, it is another burden to the patient as they potentially have to undress and must be functionally able to get a limb in and out of the water container (especially if it is on the upper thigh). In contrast, we could have tightened the inclusion criteria to burns on the forearm or lower leg but this was not considered feasible due to the timeframe of the research.

7.2.5 Study 4: Monitoring Wound Healing In Minor Burns – A Novel Approach

The comparative measure for wound healing over time was visual assessment via photographs by a Specialist Burns Consultant. Ideally a wound area measurement would have been included as another objective outcome measure. Wound area was calculated for each series of photographs using Image J, a free software package able to calculate wound area but it was not used in the final analysis (29). The wound area measure was excluded because frequently, at early follow ups, the wound margins had extended. Therefore wound area increased even though the wound was clearly healing on visual assessment. This is the nature of burn wounds and may have been due to further debridement of dead tissue with a healing wound presenting itself underneath the removed tissue. Patients who had surgery were also included in the patient cohort. If surgery was completed after recruitment the wound area had increased, due to debridement, but the wound was healing or healed. This inherently goes against a healing wound where the area decreases as it heals (30). The image J area measurement was therefore not appropriate for inclusion in the analysis. Additionally, it was difficult to account for the curve of the limb in the bigger minor limb burns, which led to large discrepancies between the two photo areas calculated. It was therefore decided to omit the image J area calculations from the analysis. Further, we were unable to examine whether there is a quantifiable volume of oedema that impacts significantly on wound healing. A degree of oedema is essential in an acute wound injury and is a normal part of the healing process (31). However, it is not certain how much oedema is detrimental to healing. It was not possible to investigate the rate of, or time to healing associated with a quantifiable volume of oedema due to the available instrumentation and equipment and the time constraints of the study.

7.3 Conclusions

The novel findings of this study demonstrate a single instrument, BIS, is capable of monitoring fluid shifts easily, in real time and in the presence of dressings in the burns population. The first study of this study series determined whole body BIS alternate electrode placement measures can be utilised in burns > 12% TBSA,

without adjustment, for the assessment of i) all resistance variables and extracellular fluid (ECF) volumes in an open wound and Acticoat^{TM} dressing, ii) total body fluid (TBF) in an open wound only. Total body fluid volumes in an Acticoat^{TM} dressing and intracellular fluid volumes in an open wound and Acticoat^{TM} dressing need to be used with caution as there is the potential for them to be over or underestimated.

The second and third studies showed BIS is a reliable method for monitoring fluid volume change across the spectrum of burns severity in any dressing condition and electrode position. Both whole body and localised BIS are accurate in the assessment of fluid shifts in major and minor burns, respectively. Silver and non-silver impregnated dressings alter BIS measures. Therefore, in the presence of dressings, BIS measures have to be corrected using the appropriate algorithms or calculator.

The final study established BIS resistance values, (R_0 ECF equivalent and R_{inf}, TBF equivalent) are able to monitor the status of minor limb burn wounds and are a useful adjunct to standard practice. However, further research is required to investigate phase angle as an indicator of the wound healing process.

7.4 Future Research

There is a plethora of opportunity to extend the use of BIS in burns as mentioned in the literature review, but in keeping with the overarching theme of this research the future recommendations will concentrate its application in the assessment of fluid volume change.

This research has demonstrated that BIS is able to monitor fluid volume change across the spectrum of burn severity with use of the developed algorithms or calculator. To make further progress and to enhance the clinical utility of BIS in the burns population, development of one workable calculator for burns greater than 5% TBSA would help achieve this. It is recommended the results of this research (Study Two) and Grisbrook et al's (2016) be pooled together to accomplish this. As touched on in Study Two, additional work is required to improve confidence in the use of BIS over standard haemodynamic monitoring in major burns for titration of resuscitation fluids. A greater understanding of the effect of large negative fluid shifts (> 100 ml) on BIS measures is also required as negative volumes of such amplitude clinically

exist. Future research design should therefore include repeated BIS measures (e.g. hourly, over seven-eight hours) over the initial 48-72 hours of burn injury, in order to capture the ebbs and flows of fluid shift in major acute burns. Study Two in this series only included five consecutive half hourly BIS measures (with dressings intact), over a two-three hour period. Ideally multi centred trials would be conducted to increase major burn patient numbers, thus providing the best representative burn population sample and generalisability of results. This would also allow for comparison of burn centre's fluid resuscitation regimes and the effects on acute burn fluid shifts. To extend BIS's ability to measure oedema change, studies need to be conducted in subacute and chronic burns to explore its reliability and validity in these sub groups.

With respect to wounds posing a potential barrier to BIS utility, further exploration into optimal alternate electrode placements is required (as discussed in Study One). A greater understanding of limb segmental measures in measuring whole body fluid volumes is also warranted. It has been suggested whole body impedance and composition may be predicted by the measurement of one extremity's (or segment of extremity) impedance (32). Bioimpedance measurement of a calf segment in dialysis patients has been shown to reflect whole body fluid shifts (33). Therefore if upper limb burns prevent the placement of hand electrodes then is it possible lower limb segmental BIS measures alone provide an option of whole body fluid volume assessment? Segmental upper and lower limb BIS measurements, in healthy individuals, were collected as a part of Grisbrook et al's study thus providing normative data (34). Limb segmental BIS measurements were also measured in study one of this series (major burns) but investigation of the results in monitoring fluid shifts were considered out of scope of the study. This data may therefore be utilised in a pilot study exploring limb segmental BIS measures in monitoring whole body fluid volume change.

An option to combine a BIS instrument with intravenous fluid pumps would enhance the utility of this non-invasive tool in not only burns, but other clinical areas where fluid resuscitation is required e.g. major trauma, severe sepsis. Combining BIS with intravenous fluid pumps would allow continuous monitoring of fluid shifts and automatic, real time titration of fluid volumes to set targets. In burns receiving fluid

resuscitation, fluid volumes delivered could be automatically titrated to maintain ICF fluid volumes within a normal average range (for given height, weight and gender) and ECF volumes within five percent of average.

Another possibility to address open wounds hindering oedema assessment by BIS is putting electrodes directly on the wound. Kekonen et al (2012) demonstrated a two electrode configuration, where one electrode was placed on the wound, was able to evaluate the status of a superficial acute wound (35). Investigation of within wound electrode configurations is worth pursuing. It eliminates the impedance of the skin and would reduce the barriers to BIS use in the burns population. Handheld microelectric, direct current generators with electrodes embedded in wound dressings have been developed to facilitate wound healing (36). These could be considered as another alternative to increase the clinical utility of BIS in populations where wounds preclude the placement of electrodes.

Furthermore, it may be more advantageous to use the change in BIS resistance values (between consecutive measures) rather than calculated volumes as it removes the need for specific predictive equations and may eliminate the need for height and weight measures (15). There are a growing number of studies, which suggest raw variables may be more useful than calculated measures in predicting clinical outcomes (15, 37). Additionally, removing the use of predictive equations has been proposed to increase the sensitivity of BIS measures to detect change (38). However, further investigation in the burns population is needed to clarify this. Future studies are also required to determine what resistance change equates to a real volume change i.e. 1 ohm = *x* ml, so an absolute volume measure can be determined without the need for predictive equations.

Following on from Study Three, research quantifying absolute volumes of oedema change over time with BIS in minor burns is also indicated. This is pertinent in oedema management interventional studies to help determine best practice. To achieve an absolute volume measure in minor burns a comparative objective measure of oedema volume change with greater sensitivity than circumference limb measures (CLM) is recommended. If funding and time allowed MRI should be considered. Water displacement volumetry (WDV) may be an option if patients with burns above the elbow and knee are excluded from the studies. If this was established the

applications of BIS wound expand considerably. With an valid technique to quantify oedema further examination of the association between oedema volume (through BIS variables) and wound healing is warranted. Improving knowledge of the effects of oedema would allow for advances in the development of treatment options to decrease and, or control oedema, of both large resuscitation and minor burns Thus further enhancing clinical decision making in the management of the patient and the burn.

Further study investigating the effect of the age of dressings on BIS measures in both localised and whole body burn wound oedema is also warranted. Dressings in acute major burns are changed every 24 - 48 hours and in minor burns they can be left in place for up to four to five days. Over time, wound exudate is absorbed by the dressing, which may potentially alter BIS measures. Therefore longitudinal studies should be conducted with BIS measures taken prior to removal of the intact dressing. A portable, mini size BIS instrument (strapped to the limb) which could monitor limb segment oedema change continuously, would allow patients to independently monitor and respond appropriately to changes in resistance measures (an index of oedema change). If resistance values significantly decreased, the patient could then instigate oedema management principles (e.g. elevation and/or movement) to reduce the oedema. A portable, real time oedema management device such as this would guide Specialist Consultant decision making and reduce the negative impact of oedema on wound healing and patient function.

The final study has demonstrated BIS as an auspicious tool in the assessment of minor burn wound healing. Additional research is indicated to examine whether PA measured at other frequencies, other than 50 KHz, are associated with minor burn wound healing. It is recommended future studies continue wound healing assessment and BIS measures until complete epithelialisation of the burn wound, rather than just an initial and follow up assessment (as per Study Four). For the purposes of research improved markers of wound healing, such as tissue sample collection for histological assessment and laser Doppler imaging, are also indicated for exploration of BIS as a wound monitoring tool. However, taking tissue samples introduce ethical issues and increase the risk of adverse outcomes for the patients (39). Another question is whether BIS variables, in major burns, are associated with the status of the wound.

Confidence in the application of BIS as method of monitoring fluid volume change in the burns environment will increase as further research is conducted and questions answered. As a result it will guide best practice in oedema management strategies and reduce the burden of burn wound injury on the individual and society.

7.5 Significance of This Research

The results of this research have demonstrated the clinical and research utility of BIS, across the spectrum of burn severity. The novel findings show BIS possesses advantages over the widely accepted and current methods of oedema measurement and wound assessment, as it is user friendly, safe, rapid and non invasive. The current findings demonstrate that bioimpedance spectroscopy can provide an immediate measure of oedema volume change, estimate resuscitation requirements and monitor wound status. It can be utilised with dressings intact, a capability WDV, CLM and wound monitoring methods do not possess. Development of algorithms (from the results of studies two and three), to adjust for the presence of dressings, further enhances the application of the instrument in this arena. The new findings from the study series may also be useful and translational to other clinical populations, such as the critically ill, traumatic limb injuries and chronic ulcers where large or minor oedema changes pose a barrier to optimal recovery and patient treatment.

Progress in optimising acute burn oedema removal has been limited by the ability to measure the efficacies of interventions. Oedema may contribute to burn wound conversion and other negative sequelae of the burn injury if urgent treatment to reduce oedema is not implemented. The results of the study series show BIS, a single instrument, has the potential to positively impact patient outcome and recovery following a burn. This will be achieved through implementation of its use immediately in patient care as tool for monitoring oedema change and through guiding future interventional studies to improve proactive oedema management and assessment of wound healing status.

“Every intervention from the point of injury influences the outcome after burn” (6).

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APPENDICES

Appendix A Declaration And Statement of Contribution By Others

This thesis contains published work and/or work prepared for publication, **which has been co-authored**. The bibliographical details of the work are presented for each study. The work involved in designing the studies described in this thesis was performed primarily by Pippa Kenworthy (candidate). The thesis outline and experimental design was planned and developed by the candidate, in consultation and assistance from Dr Tiffany Grisbrook, Dr William Gibson, Assoc Prof Dale Edgar and W. Prof. Fiona Wood (the candidate's supervisors).

All participant recruitment and management was carried out or facilitated by the candidate between December 2014 and February 2017. This was completed in association with the staff and patients of the Burn Unit and Medical Illustrations Department, initially at Royal Perth Hospital and then at Fiona Stanley Hospital (FSH) (as the burn service and unit transitioned to the new FSH February 2015). The Fiona Wood Foundation (Chevron Fellowship) has supported my clinical research time for the duration of the study.

In addition, the candidate was responsible for the data analysis with assistance from Mr Michael Phillips, biostatistician, Medical Research Fund of Royal Perth Hospital. The candidate drafted the original thesis, with Dr Tiffany Grisbrook, Dr William Gibson, Michael Phillips, Assoc Prof Dale Edgar and W. Prof. Fiona Wood providing feedback on drafts until the examinable version was finalised.

I declare that all of the material presented in this thesis is original.

28 April, 2017

Pippa Kenworthy
Senior Physiotherapist
Burns Service of Western Australia
Fiona Stanley Hospital
P: +61 08 615 72926
E: pippa.kenworthy@health.wa.gov.au

Assoc Prof Dale Edgar
Principal Supervisor
April 2017

Dr William Gibson
Co- supervisor
April 2017

W. Prof Fiona Wood
Co- supervisor
April 2017

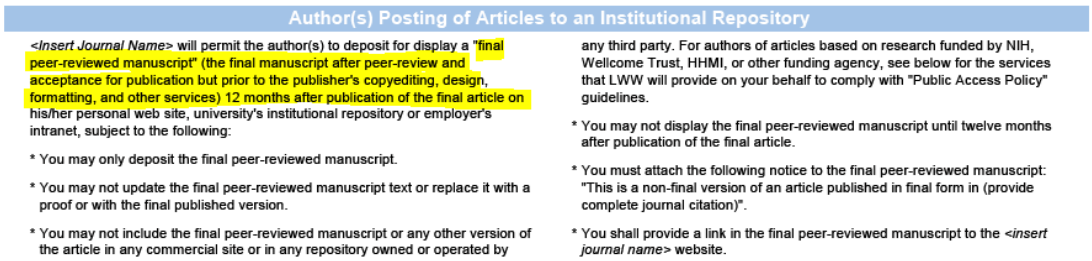
Dr Tiffany Grisbrook
Co- supervisor
April 2017

Michael Phillips
Biostatistician
April 2017

Appendix B Letter of Approval from Publishers

Dear Pippa,

Please see the below snip of our Copyright Transfer Agreement, signed by our authors upon publishing in WK/LWW journals:



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<Insert Journal Name> will permit the author(s) to deposit for display a "final peer-reviewed manuscript" (the final manuscript after peer-review and acceptance for publication but prior to the publisher's copyediting, design, formatting, and other services) 12 months after publication of the final article on his/her personal web site, university's institutional repository or employer's intranet, subject to the following:

* You may only deposit the final peer-reviewed manuscript.
* You may not update the final peer-reviewed manuscript text or replace it with a proof or with the final published version.
* You may not include the final peer-reviewed manuscript or any other version of the article in any commercial site or in any repository owned or operated by any third party. For authors of articles based on research funded by NIH, Wellcome Trust, HHMI, or other funding agency, see below for the services that LWW will provide on your behalf to comply with "Public Access Policy" guidelines.
* You may not display the final peer-reviewed manuscript until twelve months after publication of the final article.
* You must attach the following notice to the final peer-reviewed manuscript: "This is a non-final version of an article published in final form in (provide complete journal citation)".
* You shall provide a link in the final peer-reviewed manuscript to the *<insert journal name>* website.

To reiterate: you may include the *final peer-reviewed version* of this article (**Addressing the barriers to bioimpedance spectroscopy use in major burns: alternate electrode placement**) in your institutional repository, 12 months after the publication of your article in *JBC&R*. In your case, the embargo will lift in March 2018.

All best,
Theresa

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A link to the Copyright Transfer Agreement authors sign on submission of manuscript to *Journal of Burn Care and Research* can be found here. file:///Users/pippakenworthy/Downloads/eCTA_Journal%20Burn%20Care%20Research_2017.pdf

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Dear Pippa,

re: **Bioimpedance spectroscopy: A technique to monitor interventions for swelling in minor burns**. *Journal of Burns*

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Best of luck with your dissertation and best regards,
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Laura Stingelin
Permissions Helpdesk Associate
ELSEVIER | Global E-Operations Books
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l.stingelin@elsevier.com
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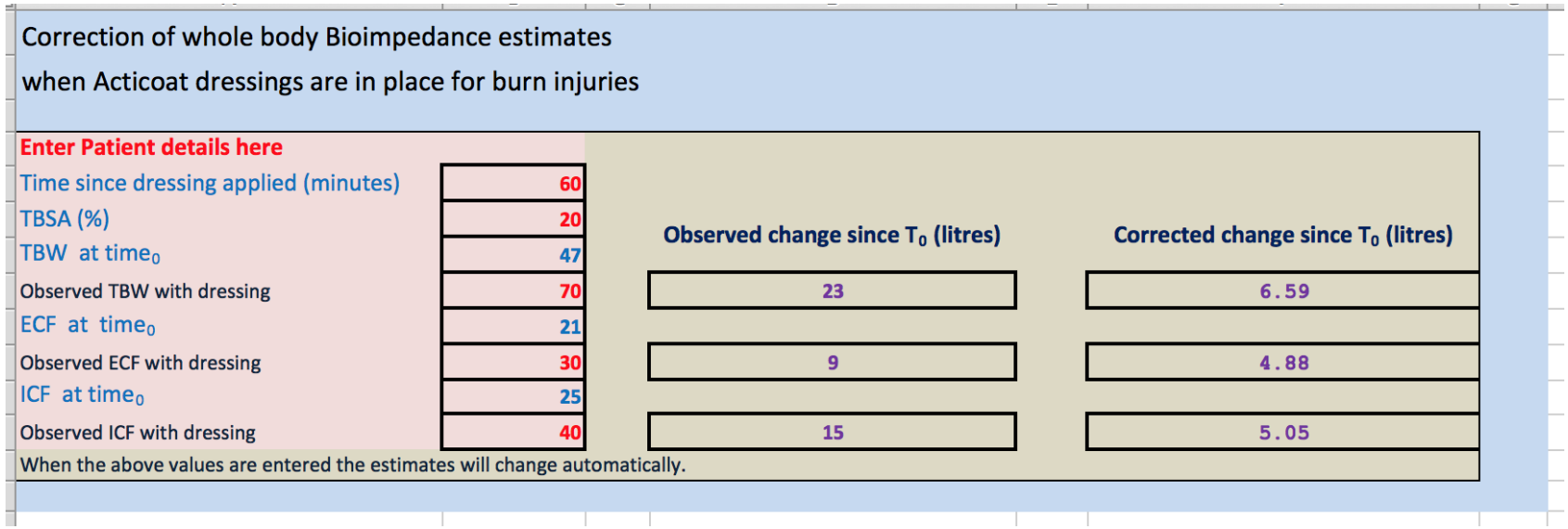
Appendix C Acticoat Calculator for Oedema (screen shot)



Correction of whole body Bioimpedance estimates
when Acticoat dressings are in place for burn injuries

Enter Patient details here			
Time since dressing applied (minutes)	60		
TBSA (%)	20	Observed change since T_0 (litres)	Corrected change since T_0 (litres)
TBW at $time_0$	47		
Observed TBW with dressing	70	23	6.59
ECF at $time_0$	21		
Observed ECF with dressing	30	9	4.88
ICF at $time_0$	25		
Observed ICF with dressing	40	15	5.05

When the above values are entered the estimates will change automatically.

*workable excel version submitted as supplementary material in the Burns and Trauma Journal.

Appendix D Example Of Study 1 & 2 Completed Data Collection Sheet

Subject Number: 12 Subject Initials: AP.

BAB__ ID:

DATE OF EXAMINATION

Date Baseline Measure: ____/____/____ (dd / mm / yy) 12/4/15

DEMOGRAPHY

Gender: ☐ Male ☑ Female

Date of Birth: 12/11/79. 36yrs.

Country of Birth: —

Ethnicity: ☐ Caucasian ☑ Aboriginal/Torres Strait Islander ☐ Asian ☐ Other, please specify _____

Dominant side Left / Right *(please circle)*

Occupation: unemployed

Workers Compensation: ☐ Yes ☐ No

Date of Injury: 10-11/4/15 (dd / mm / yy)

Time of Injury: ~~0~~ around midnight.

Date of Admission: 11/4/15. (dd / mm / yy)

Time of Admission: 0200hrs (11/4)

Alcohol pre burn: Yes No In excess?

Circumstances of Injury: ☐ Accident at work ☑ Non-work accident ☐ Assault ☑ Self Harm

Comment: Escharotomy ⓛ UL 11/4/15.

Burn Agent: self immolation — petrol.

% TBSA: 25.

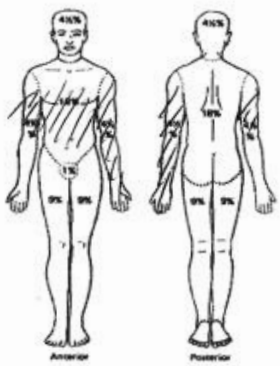
Depth of Burn

☐ Superficial

☐ Superficial Partial

☑ Deep Partial

☑ Full Thickness



(Please mark the location)

Burns Service of WA EC2011/028
W:\Burns\RPH\Telstra Burn Outcomes Centre\Bioimpedance - Pippa Kenworthy\BIS Recruitment Pack\Localised SOP\1 stop shop whole body BIS

Subject Number: 12 Subject Initials: AP.

BAB__ ID:

Whole Body BIA >20% TBSA

Date: 12/4/15 Time: 0910 Burn Date: 11/24/15 Burn Time: _____

Calibration Check: Y N

% burn 25. Depth of burn DPT-FT Burn Location bilat UL's chest, face.

Weight: 51.6 kg Height: 160 m

Room Temperature: _____

Initial/Pre-Ax Vital Signs

BP	PR (per min)	SpO2 %	Temp	Resp. Rate (per min)

Pre measure fluid balance: ⊕/- 2258 **ml**

Date: 12/4/15 **Time:** 0910.

No Dressing	**Measure 1**	**Measure 2**	**Measure 3**
Standard Electrode Placement w0nd__stwb	348	349	350
Right upper limb Standard w0nd__stra	—		3
Right lower limb Standard w0nd__strl	366	367	368. ~~357~~
Alternate Electrode Placement w0nd__alwb	363	364	365
Right upper limb Alternate w0nd__alra	—		
Right lower limb Alternate w0nd__alrl	360	361	362

__= subject number

Subject Number: 12 Subject Initials: AR

BAB__ ID:

Dressing Type and location: Acticoat, flamingo

New Dressing

Time 1 (Time: 9010)			
Time (1/2 hrly)	**Measure 1**	**Measure 2**	**Measure 3**
Standard Electrode Placement w1d_stwb	369	370	371
Right upper limb Standard w1d_stra			
Right lower limb Standard w1d_strl	372	373	374
Alternate Electrode Placement w1d_alwb	378	379	380
Right upper limb Alternate w1d_alra			
Right lower limb Alternate w1d_alrl	375	376	377
Time 2 (Time: 1038)			
Standard Electrode Placement w2d_stwb	381	382	383
Right upper limb Standard w2d_stra			—
Right lower limb Standard w2d_strl	384	385	386
Time 3 (Time: 1102)			
Standard Electrode Placement w3d_stwb	390	391	392
Right upper limb Standard w3d_stra			—
Right lower limb Standard w3d_strl	387	388	389
Time 4 (Time: 1132)			
Standard Electrode Placement w4d_stwb	396	397	398
Right upper limb Standard w4d_stra			—
Right lower limb Standard w4d_strl	393	394	395
Time 5 (Time: 1156)			
Standard Electrode Placement w4d_stwb	402	403	404
Right upper limb Standard w4d_stra	—		—
Right lower limb Standard w4d_strl	0399	400	401

Appendix E Example Of Study 3&4 Completed Data Collection Sheet

Subject Number: 12 Subject Initials: V R

BABSLocal Number:

Localised BIS

Date: 7/4 **Time:** 1010. **Burn Date:** Fri 3/4/15 ~1230pm.

Calibration Check: Y (N) **DoB:** _____ 46yo

% TBSA burn 0.75 **% burn affected area** 0.75

Depth of burn SPT **Burn Location** (R) distal volar forearm

Weight: 70 **kg** **Height:** 156 **m**

Limb: (R) L (UL) LL **Hand Dominance:** (R) L

Distance between localised sense electrodes (3cm either side of wound): 14 **cm**

Localised sense electrode placement	Proximal (white)	Distal (blue)
Landmark (bony anatomical)	11 cm prox	3cm distal
Distance Electrode – landmark (cm)	Bleeo ulnar	styloid.

- Flame + scald burn.
- Occ: streaks/skelves, greasy filler.

Subject Number: 12 Subject Initials: VR

BABSLocal Number:

First Measure (Post-burn day 4) ~~10~~

Date: 7/4/15 **Time:** 10:15

Circumference	Proximal (cm)	Distal (cm)	Mid point (cm)
Affected limb	19.5	17 (at wrist)	18
Unaffected limb	~~22.0~~ 19.3. R/K	15.5	18.

The width of the tape measure to be kept distally to the point of measure.

No Dressing	**Measure 1**	**Measure 2**	**Measure 3**
Whole Body BIA Lnd__wb	0213	0214	215.
Localised L nd__lo	0198.	0199	0200
Affected limb Segment Lnd__sa, a/l	0201	0202	0203
Unaffected limb segment Lnd__su, a/l	0210	0211	0212

__=subject number, a/l=arm or leg

New Dressing Type: duoderm.	**Measure 1**	**Measure 2**	**Measure 3**
Whole Body BIA Ld__wb	0222	0223	~~218~~ 224
Localised Ld__lo	0228	0229	230
Affected Limb Segment Ld__sa, a/l	0225	0226	~~221~~ 227
Unaffected limb segment Ld__su, a/l	0231	0232	233

__=subject number, r/l=right or left, a/l=arm or leg

Wound Healing, Visitrak area measure: _____cm2

Subject Number: 12 Subject Initials: VR.

BABSLocal Number:

Second Measure (Post-burn day 7) **Weight (kg)** 70

Date: 10/4 **Time:** 0830 **Surgery: Y(N), date:**

Circumference	Proximal (cm)	Distal (cm)	Mid (cm)
Affected limb	19.5	17.5	17.0
Unaffected limb	~~15~~ 20	~~20~~ 15 wrist	16

No Dressing	Measure 1	Measure 2	Measure 3
Whole Body BIA Lnd__wb	318	319	320
Localised L nd__lo	324	325	326
Affected limb Segment Lnd__sa, a/l	321	322	323
Unaffected limb segment Lnd__su, a/l	327	328	329

__=subject number, r/l=right or left, a/l=arm or leg

New Dressing Type: Algisite + fix	Measure 1	Measure 2	Measure 3
Whole Body BIA Ld__wb	330	331	332
Localised Ld __lo	336	337	338
Affected Limb Segment Ld__sa, a/l	333	334	335
Unaffected limb segment Ld__su, a/l	339.	340	~~338~~ 341

__=subject number, r/l=right or left, a/l=arm or leg

Wound Healing, Visitrak area measure (<1% TBSA): _____

Appendix F Consent Form, Study 1 & 2

FIONA STANLEY HOSPITAL

CONSENT FORM

I,..... *(block letters)* agree to take part in the research study:

Using bioimpedance spectroscopy to monitor acute burn swelling of the limbs.

I understand the aim of this research study is to find the best placement for electrodes to measure fluid distribution and swelling around the burn. I consent to participate in this project, the details of which have been explained to me, and I have been provided with a written information sheet to keep.

I understand that my participation will involve a screening survey, and the use of a bioimpedance machine and circumference measures. I understand that I will need to expose my arms and legs in order to participate in the study. I agree that the researcher may use the results as described in the information sheet.

I acknowledge that:

- taking part in this study is voluntary and I am aware that I can stop taking part in it at any time without explanation or prejudice and to withdraw any unprocessed data I have provided;
- that any information I give will be kept strictly confidential and that no names will be used to identify me with this study without my approval;
- Any risks and possible effects of the Bioimpedance Machine has been explained to my satisfaction;

(Please tick to indicate consent)

I consent to have my height and weight measured	☐	**Yes**	☐	**No**
I consent to have bioimpedance to measure my whole body and each of the affected and unaffected limbs	☐	**Yes**	☐	**No**
I consent to have a photo taken of my burn wound	☐	**Yes**	☐	**No**

This research study adheres to the guidelines of the ethical review process of Royal Perth Hospital. Whilst you are free to discuss your participation in this study with research staff (contactable on 0413 070 384), if you would like to speak to an officer of the hospital not involved in the study, you may contact the Prof Frank Bockxmeer, Chairman of the RPH Ethics Committee on (08) 9224 2292.

Participant:..... Date:.....
(Signature)

Witness:..... Date:.....
(Signature)

I have explained the nature and purpose of the study to the above participant and have answered all their questions.

Researcher:.....Date:.....
(Signature)

I would like to receive a summary of the completed research outcomes: Yes No

FSH Localised BIS consent, version 1 dated 09/03/2015. EC 2011/028, based on Master RPH Localised BIS consent, version 2. EC 2011/028

Appendix G Consent Form, Study 3 & 4

FIONA STANLEY HOSPITAL

CONSENT FORM

I,..... *(block letters)* agree to take part in the research study:

Using bioimpedance spectroscopy to monitor acute burn swelling of the limbs in large burns.

I understand the aim of this research study is to find the best placement for electrodes to measure fluid distribution and swelling around the burn. I consent to participate in this project, the details of which have been explained to me, and I have been provided with a written information sheet to keep.

I understand that my participation will involve a screening survey, and the use of a bioimpedance machine and circumference measures. I understand that I will need to expose my arms and legs in order to participate in the study. I agree that the researcher may use the results as described in the information sheet.

I acknowledge that:

- taking part in this study is voluntary and I am aware that I can stop taking part in it at any time without explanation or prejudice and to withdraw any unprocessed data I have provided;
- that any information I give will be kept strictly confidential and that no names will be used to identify me with this study without my approval;
- Any risks and possible effects of the Bioimpedance Machine has been explained to my satisfaction;

(Please tick to indicate consent)

I consent to have my height and weight measured.	☐	**Yes**	☐	**No**
I consent to have bioimpedance to measure my whole body with 2 sets of different electrode placements.	☐	**Yes**	☐	**No**
I consent to have 5 sets of bioimpedance measures (as above) over a period of 3-4 hours.	☐	**Yes**	☐	**No**

This research study adheres to the guidelines of the ethical review process of Royal Perth Hospital. Whilst you are free to discuss your participation in this study with research staff (contactable on 0413 070 384), if you would like to speak to an officer of the hospital not involved in the study, you may contact the Prof Frank Bockxmeer, Chairman of the RPH Ethics Committee on (08) 9224 2292.

Participant:..... Date:.....
(Signature)

Witness:..... Date:.....
(Signature)

I have explained the nature and purpose of the study to the above participant and have answered all their questions.

Researcher:.....Date:.....
(Signature)

I would like to receive a summary of the completed research outcomes: Yes No

FSH Whole Body BIS, version 1 dated 09/03/2015, based on Master RPH Whole Body BIS, Version 2 EC2011/028

Appendix H Plain Language Statement, Study 1 & 2

FIONA STANLEY HOSPITAL

Participant Information Sheet

Using bioimpedance spectroscopy to monitor acute burn swelling.
Ethical Approval Number: 2011/028

Chief Investigator: Dr Dale W. Edgar
Senior Physiotherapist, Burn Service, Fiona Stanley Hospital

Co-investigators:
Pippa Kenworthy – Senior Physiotherapist, Burn Service, FSH
Dr Tiffany Grisbrook – Research Fellow, Fiona Wood Foundation
Dr William Gibson, School of Physiotherapy, The University of Notre Dame Australia
W.Prof Fiona Wood – Director, Burn Service of WA

Do you have a pacemaker?	Yes [ ]	No [ ]
Are you pregnant?	Yes [ ]	No [ ]

If "Yes" to either of the above: You will be unable to participate in this study. Thank you for your time and interest.

You are invited to take part in this research study and this sheet explains briefly what is involved. Please read it carefully and a researcher will be available to answer any questions before you decide to take part.

AIMS AND SIGNIFICANCE OF THE STUDY

A burn is an injury to the body tissue that is the result of heat, electricity, radiation, or chemicals. In Australia and New Zealand ~1% of people will suffer a burn each year. Most often it is the skin that sustains the damage, however, in severe burns, the muscle, fat and bone may also be affected. Following an injury caused by a burn, the tissue will swell as fluid leaks from the surrounding blood vessels. Swelling often remains for up to 5 days after the injury. The movement, or loss of body fluid, that occurs is known as a 'fluid shift'. The management of fluid shifts involves fluid replacement or resuscitation, occasionally supplied directly into the bloodstream via an IV drip. The adjustment of fluid requirements for severe burns is dynamic. Close monitoring is required to prevent complications, especially in the first 24-48 hours. When treating a burn, the doctor treads a fine line between excess tissue swelling, which slows wound healing and increases the risk of scar; and the prevention of shock, kidney failure and possibly death.

The current methods for measuring fluid shifts are invasive and lack precision, especially when measuring limb swelling. In 2009, researchers at the Royal Perth Hospital (RPH) burn unit demonstrated the usefulness of a non-invasive device, known as bioimpedance spectroscopy (BIS) for measuring fluid changes after burns. However, BIS is hindered by the presence of open wounds, especially on the hands and feet of the patient. One such issue is whether the type and presence of a wound and the dressing, in a region measured by BIS,

will affect the accuracy of the method. To answer this question we require your assistance, so that we may take BIS measurements before, during and after dressing changes so that we may compare these with the known fluid shifts during that time.

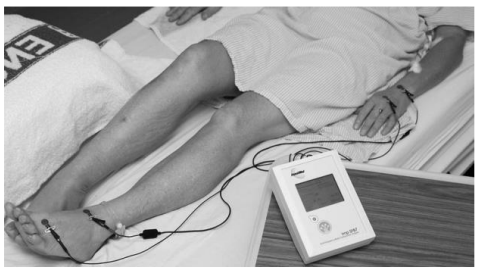
YOUR INVOLVEMENT

BIS Procedure

On the first day of your involvement you will have your height and weight measured and your limb length and circumferences recorded whilst clothed. Six sets of BIS measurements of your whole body will be taken at intervals of thirty minutes, over the course of two hours. One set with your dressings removed and five sets after your dressing change. Each set of measures takes less than one minute, is painless and only requires that you do not move for that period. Your vital signs and urine output will also be recorded.

BIS is a technique that is commonly used by dieticians and fitness trainers to estimate fat and lean tissue in the body. It is based on the principle that impedance to the flow of an electric current through the body is directly related to the amount of water in the body. In our study, however, we are using the measurement of impedance in the body and the limbs to estimate the amount of water and obtain a measure of swelling volume.

The impedance instrument to be used is commercially available, meets all relevant safety standards and is in wide use throughout Australia. A small electric current is applied to the body via electrodes and the impedance to the flow of current is measured (see picture). The current is a very small AC current from a battery-powered instrument. The current is so small you will not feel it and it will be of no danger to you. You will be asked to lie on your bed and the self-adhesive gel electrodes placed on the skin surface on the hand, wrist, elbow, knee, ankle and toes.



RISKS AND POTENTIAL BENEFITS OF THE STUDY

Infection: All equipment will be cleaned according to standard hospital procedures adhered to by the Fiona Stanley Burns Unit. All gel adhesive electrodes are single-use and will be placed on intact skin and will not come into contact with your open wound, thus posing only minimal risk of infection.

Injury: The BIS device is battery operated. The current from which the resistance measurements are derived is very small. The machine has been tested previously on patients and staff in the FSH Burn Unit without any injury or ill effect noted.

There will be no direct benefit for you from participation in the study, however your participation may benefit burns patients in future.

This study is part of a Masters research study being undertaken by Ms Pippa Kenworthy, and is supervised by Dr Dale Edgar. The Masters is being undertaken through the School of Physiotherapy at the University of Notre Dame Australia, Fremantle, WA. Dr William Gibson from the School of Physiotherapy is also supervising Ms Kenworthy.

COST OF PARTICIPATION

There is no cost to you associated with participating.

ACTION IF ADVERSE EVENT OCCURS DURING STUDY

In the event that you suffer an adverse event or a medical accident during this study that arises from the participation in the study, you will be offered all full and necessary treatment by Fiona Stanley. The Royal Perth Hospital Ethics Committee has approved this study on the basis (amongst others) that the reported risk of such an event is either small or acceptable in terms of the risk you face as a result of your current illness or the benefit that is possible with the new treatment being tested. No provisions have been made in this trial to offer trial subjects who suffer adverse reaction monetary compensation, but the absence of such a provision does not remove your right to seek compensation under common law.

PRIVACY AND CONFIDENTIALITY

The information gathered about you during the study will be held by the investigator in strict confidence. The study will not keep any data after the research has been completed. All data will be stored in a computer in the FSH with access via a password known only to investigators. All data collection sheets will be stored in a locked filing cabinet for a period of seven years, as required by law.

Your trial records (without your name attached) will be made available to government regulatory bodies in Australia if required. All people who handle your information will adhere to traditional standards of confidentiality and will comply with all relevant privacy legislation. In Australia, this is in the Privacy Act 1988. The Ethics Committee has obtained assurances from the sponsor that the 'Information Privacy Principles' laid down in the Act will be met, and will oblige the investigator and other hospital staff to meet strict privacy standards. The Privacy Act does not apply overseas but there is equivalent binding legislation force in the USA, the European Union and elsewhere. If the results of the trial are published in a medical journal, as is intended, no reader will be able to identify individual patients.

REQUESTS FOR MORE INFORMATION

The investigators encourage you to discuss any questions or concerns regarding the study with them at any time throughout the study. The chief investigator for this study if Dr Dale Edgar, who can be contacted on 0413070384 or email dale.edgar@health.wa.gov.au. This research project has been approved by the Ethics Committee at Royal Perth Hospital and the University of Notre Dame Australia (approval number 014139F). If you have any questions about your rights as a research participant, please contact Assoc Prof Frank van Bockxmeer, Chairman of the Ethics Committee, on (08) 9224 2244. Alternatively if you wish to make a complaint regarding the manner in which this research project is conducted, it should be directed to the Executive Officer of the Human Research Ethics Committee,

THE UNIVERSITY OF NOTRE DAME AUSTRALIA

Research Office, the University of Notre Dame Australia, PO Box 1225, Fremantle WA 6959, phone (08) 9433 0943, research@nd.edu.au

You will be asked to sign a consent form before you take part but you are free to withdraw at any stage of the proceedings without prejudice to any future medical care.

It will be possible to access a summary of the completed research on the Fiona Wood Foundation website at www.**fionawoodfoundation**.com

Thank you for your participation.

Appendix I Plain Language Statement, Study 3 & 4

Fiona Stanley Hospital

Participant Information Sheet

Using bioimpedance spectroscopy to monitor acute burn swelling.
Ethical Approval Number: 2011/028

Chief Investigator: Dr Dale W. Edgar
Senior Physiotherapist, Burn Service, Fiona Stanley Hospital

Co-investigators:
Pippa Kenworthy – Senior Physiotherapist, Burn Service, FSH
Dr Tiffany Grisbrook – Research Fellow, Fiona Wood Foundation
Dr William Gibson, School of Physiotherapy, The University of Notre Dame Australia
W.Prof Fiona Wood – Director, Burn Service of WA

Do you have a pacemaker?	Yes [ ]	No [ ]
Are you pregnant?	Yes [ ]	No [ ]

If "Yes" to either of the above: You will be unable to participate in this study. Thank you for your time and interest.

You are invited to take part in this research study and this sheet explains briefly what is involved. Please read it carefully and a researcher will be available to answer any questions before you decide to take part.

AIMS AND SIGNIFICANCE OF THE STUDY

A burn is an injury to the body tissue that is the result of heat, electricity, radiation, or chemicals. In Australia and New Zealand ~1% of people will suffer a burn each year. Most often it is the skin that sustains the damage, however, in severe burns, the muscle, fat and bone may also be affected. Following an injury caused by a burn, the tissue will swell as fluid leaks from the surrounding blood vessels. Swelling often remains for up to 5 days after the injury. The movement, or loss of body fluid, that occurs is known as a 'fluid shift'. The management of fluid shifts involves fluid replacement or resuscitation, occasionally supplied directly into the bloodstream via an IV drip. The adjustment of fluid requirements for severe burns is dynamic. Close monitoring is required to prevent complications, especially in the first 24-48 hours. When treating a burn, the doctor treads a fine line between excess tissue swelling, which slows wound healing and increases the risk of scar; and the prevention of shock, kidney failure and possibly death.

The current methods for measuring fluid shifts are invasive and lack precision, especially when measuring limb swelling. In 2009, researchers at the Royal Perth Hospital (RPH) burn unit demonstrated the usefulness of a non-invasive device, known as bioimpedance spectroscopy (BIS) for measuring fluid changes after burns. However, BIS is hindered by the presence of open wounds, especially on the hands and feet of the patient. One such issue is whether the type and presence of a wound and the dressing, in a region measured by BIS, will affect the accuracy of the method. To answer this question we require your assistance,

so that we may take BIS measurements before, during and after dressing changes so that we may compare these with the known fluid shifts during that time.

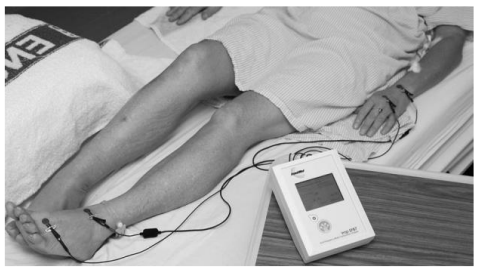
YOUR INVOLVEMENT

BIS Procedure

On the first day of your involvement you will have your height and weight measured and your limb length and circumferences recorded whilst clothed. Six sets of BIS measurements of your whole body will be taken at intervals of thirty minutes, over the course of two hours. One set with your dressings removed and five sets after your dressing change. Each set of measures takes less than one minute, is painless and only requires that you do not move for that period. Your vital signs and urine output will also be recorded.

BIS is a technique that is commonly used by dieticians and fitness trainers to estimate fat and lean tissue in the body. It is based on the principle that impedance to the flow of an electric current through the body is directly related to the amount of water in the body. In our study, however, we are using the measurement of impedance in the body and the limbs to estimate the amount of water and obtain a measure of swelling volume.

The impedance instrument to be used is commercially available, meets all relevant safety standards and is in wide use throughout Australia. A small electric current is applied to the body via electrodes and the impedance to the flow of current is measured (see picture). The current is a very small AC current from a battery-powered instrument. The current is so small you will not feel it and it will be of no danger to you. You will be asked to lie on your bed and the self-adhesive gel electrodes placed on the skin surface on the hand, wrist, elbow, knee, ankle and toes.



RISKS AND POTENTIAL BENEFITS OF THE STUDY

Infection: All equipment will be cleaned according to standard hospital procedures adhered to by the Fiona Stanley Burns Unit. All gel adhesive electrodes are single-use and will be placed on intact skin and will not come into contact with your open wound, thus posing only minimal risk of infection.

Injury: The BIS device is battery operated. The current from which the resistance measurements are derived is very small. The machine has been tested previously on patients and staff in the FSH Burn Unit without any injury or ill effect noted.

There will be no direct benefit for you from participation in the study, however your participation may benefit burns patients in future.

This study is part of a Masters research study being undertaken by Ms Pippa Kenworthy, and is supervised by Dr Dale Edgar. The Masters is being undertaken through the School of Physiotherapy at the University of Notre Dame Australia, Fremantle, WA. Dr William Gibson from the School of Physiotherapy is also supervising Ms Kenworthy.

COST OF PARTICIPATION

There is no cost to you associated with participating.

ACTION IF ADVERSE EVENT OCCURS DURING STUDY

In the event that you suffer an adverse event or a medical accident during this study that arises from the participation in the study, you will be offered all full and necessary treatment by Fiona Stanley. The Royal Perth Hospital Ethics Committee has approved this study on the basis (amongst others) that the reported risk of such an event is either small or acceptable in terms of the risk you face as a result of your current illness or the benefit that is possible with the new treatment being tested. No provisions have been made in this trial to offer trial subjects who suffer adverse reaction monetary compensation, but the absence of such a provision does not remove your right to seek compensation under common law.

PRIVACY AND CONFIDENTIALITY

The information gathered about you during the study will be held by the investigator in strict confidence. The study will not keep any data after the research has been completed. All data will be stored in a computer in the FSH with access via a password known only to investigators. All data collection sheets will be stored in a locked filing cabinet for a period of seven years, as required by law.

Your trial records (without your name attached) will be made available to government regulatory bodies in Australia if required. All people who handle your information will adhere to traditional standards of confidentiality and will comply with all relevant privacy legislation. In Australia, this is in the Privacy Act 1988. The Ethics Committee has obtained assurances from the sponsor that the 'Information Privacy Principles' laid down in the Act will be met, and will oblige the investigator and other hospital staff to meet strict privacy standards. The Privacy Act does not apply overseas but there is equivalent binding legislation force in the USA, the European Union and elsewhere. If the results of the trial are published in a medical journal, as is intended, no reader will be able to identify individual patients.

REQUESTS FOR MORE INFORMATION

The investigators encourage you to discuss any questions or concerns regarding the study with them at any time throughout the study. The chief investigator for this study if Dr Dale Edgar, who can be contacted on 0413070384 or email dale.edgar@health.wa.gov.au. This research project has been approved by the Ethics Committee at Royal Perth Hospital and the University of Notre Dame Australia (approval number 014139F). If you have any questions about your rights as a research participant, please contact Assoc Prof Frank van Bockxmeer, Chairman of the Ethics Committee, on (08) 9224 2244. Alternatively if you wish to make a complaint regarding the manner in which this research project is conducted, it should be directed to the Executive Officer of the Human Research Ethics Committee,

THE UNIVERSITY OF NOTRE DAME AUSTRALIA

Research Office, the University of Notre Dame Australia, PO Box 1225, Fremantle WA 6959, phone (08) 9433 0943, research@nd.edu.au

You will be asked to sign a consent form before you take part but you are free to withdraw at any stage of the proceedings without prejudice to any future medical care.

It will be possible to access a summary of the completed research on the Fiona Wood Foundation website at www.**fionawoodfoundation**.com

Thank you for your participation.

Appendix J Fiona Stanley Hospital Governance Approval

Government of **Western Australia**
Department of **Health**

FIONA STANLEY hospital

Our ref : Trial No 2014-106 approval FSH
Enquiries : 6152 2592

Dr Dale Edgar
Burns Unit
Fiona Stanley Hospital
102-118 Murdoch Drive
MURDOCH WA 6149

Dear Dr Edgar

FSH Project No: 2014-106
HREC No: 2011/028
Project Title: "Clinical translation of bioimpedance spectroscopy for monitoring the impact of interventions for acute burn swelling".
Protocol No: Standardised Protocol Localised BIS Version 2

On behalf of Fiona Stanley Hospital, I give authorisation for your research project to be conducted at Fiona Stanley Hospital.

The following site specific documents are to be used in addition to those approved by the Human Research Ethics Committee (HREC) in the letter dated 3 February 2015.

Document
Fiona Stanley Hospital Site Participant Information Sheet and Consent Form Whole Body BIS Version 1.0 dated 09 March 2015 based on Master RPH PICF Whole Body BIS Version 2
Fiona Stanley Hospital Site Participant Information Sheet and Consent Form Localised BIS PLS Version 1.0 dated 09 March 2015 based on Master RPH Localised BIS PLS PICF Version 2

This authorisation is based on the approval from the Royal Perth Hospital Human Research Ethics Committee and the review from the Research Governance Office. This authorisation is valid subject to the ongoing approval from the HREC, and on the basis of compliance with the 'Conditions of Site Authorisation to Conduct a Research Project' (attached) and with the compliance of all reports as required by the Research Governance Office and approving HREC. Noncompliance with these requirements could result in the authorisation be withdrawn.

The responsibility for the conduct of this project remains with you as the Principal Investigator at the site.

Yours sincerely

Dr Robyn Lawrence
EXECUTIVE DIRECTOR

9 March 2015

Att

102-118 Murdoch Drive
Murdoch WA 6149
Telephone: (08) 6152 2222

Locked Bag 100
Palmyra DC, WA 6961
www.fsh.health.wa.gov.au

Appendix K HREC Approval

Department of Health
Government of Western Australia
South Metropolitan Area Health Service

Royal Perth Hospital

ETHICS COMMITTEE

Frank M van Bockxmeer PhD MHGSA, FAHA, FFSc(RCPA)
Professor of Pathology and Laboratory Medicine
The University of Western Australia

Ethics Office
Room 5105 Level, 5 Colonial House
Tel: 9224 2292; Fax: 9224 3688

Ref: EC 2011/028
(This number must be quoted on all correspondence)

4th April 2011

Dale Edgar
Burns Service of WA
Royal Perth Hospital

Dear Dale

2011/028 Clinical Translation of Bioimpendance Spectroscopy for Monitoring the impacts of Interventions for Acute Burn Swelling

Thank you for your responses to the queries raised by the Ethics Committee and I am pleased to advise that the above study is now **APPROVED.**

The following general conditions apply to all approvals by this Committee, and starting a trial or research project following the issue of ethics approval will be deemed to be an acceptance of them by all investigators:

1. The submission of an application for Ethics Committee approval will be deemed to indicate that the investigator and any sponsor recognises the Committee as a registered (with AHEC) Health Research Ethics Committee and that it complies in all respects with the National Statement on Ethical Conduct Research Involving Humans and all other national and international ethical requirements. **The Committee will not enter into further correspondence on this point.**

2. All income arising from the study must be lodged in a hospital special purposes account. Performance of a clinical trial for a sponsor is a service for tax purposes and all GST obligations must be met.

3. The investigator will report adverse events accompanied by a statement as to whether or not the trial should continue. The Committee reserves the right to not receive reports whose complexity or level of detail requires the expenditure of unreasonable time and effort. The Committee receives voluminous paperwork relating to adverse event reporting. From time to time the Committee chairman may require these reports to be summarised and approval is granted subject to the agreement of the investigator that he or she will prepare such a summary on request.

4. The Committee has decided that, as the responsibility for the conduct of trials lies with the investigator, all correspondence should be signed by the investigator.

5. All trial drugs must be dispensed by the Pharmacy Department. A fee is levied for this service and investigators must regard this fee as an item requiring a budget allocation. Alternatively, if a sponsor agrees, separate direct funding of pharmacy services may be undertaken. There are provisions for this fee to be waived for locally-inspired unfunded studies not having an external sponsor.

6. Though state institutions are outside the jurisdiction of the Privacy Act and related legislation, the Committee will assume that the privacy provisions of that Act will be the minimum standards applying during the conduct of a trial at Royal Perth Hospital. Traditional standards of patient confidentiality will apply.

7. The Committee will not acknowledge trial communications as a matter of course, unless they relate to a matter requiring Committee approval. Evidence of dispatch of a letter will be deemed to be evidence of receipt. This rule may be waived at the Committee's discretion on provision of a *pro forma* receipt by the investigator for the Chairman's signature and return. However, trivial correspondence (as judged by the Committee) will not be acknowledged even if a *pro forma* receipt is provided. Where an investigator requests written approval or written record of a matter for special purposes (say at the request of a sponsor), the investigator should prepare the required letter for the chairman's signature rather than expect the Committee secretary to prepare it. This mechanism increases the probability that the trial details in the letter are correct.

8. The Committee will provide the names and representative affiliation of members on request, but will not provide personal details or voting records.

9. A brief annual report on each project approved will be required at the end of each fiscal year, in default of which approval for the study may be suspended. Ethics approvals at RPH do not carry an expiry date so the annual report is an important part of Ethics Committee procedure.

10. The Committee has the authority to audit the conduct of any trial without notice. Exercise of this authority will only be considered if there are grounds to believe that some irregularity has occurred or if a complaint is received from a third party, or the Committee wishes to undertake an audit for QA purposes.

11. Complaints relating to the conduct of a clinical trial should be directed to the Chairman and will be promptly investigated. Complaints about the Ethics Committee decisions or policies that cannot be resolved by discussion with the Chairman or about any actions of a particular member including the Chairman, should be directed to the Director of Clinical Services. Only written complaints (not e-mail) will be accepted for investigation.

Investigators of sponsored studies are advised to draw the above conditions to the attention of the sponsor. Investigators are reminded that records of consent or authorisation for participation in special studies (including clinical trials) form part of the Acute Hospital Patient Record and should be stored with that record in accordance with the WA *Health Patient Information Retention and Disposal Schedule (Version 2) 2000.* A copy of the 'Patient Information Sheet' should also be included in the medical records as part of informed consent documentation.

Yours sincerely

Prof Frank M van Bockxmeer
Chairman, Royal Perth Hospital Ethics Committee

The Royal Perth Hospital Ethics Committee is constituted and operates in accordance with NH&MRC Guidelines.

Appendix L HREC Letter For Additional Site

Government of Western Australia
Department of Health
South Metropolitan Health Service

Royal Perth Hospital

HUMAN RESEARCH ETHICS COMMITTEE

3 February 2015

Dr Dale Edgar
Burns Unit
Royal Perth Hospital

Dear Dale

Project Title: **Clinical Translation of Bioimpendance Spectroscopy for Monitoring the impacts of Interventions for Acute Burn Swelling**
HREC Reference: **EC 2011/028**

I am writing to confirm the **addition of the following site/s** to this research project:

Site
Fiona Stanley Hospital

Site Principal Investigator (PI)
Dr Dale Edgar

The following **documents** have been approved for use in this project:

Localised BIS Study
- Standardised Protocol localised BIS, V2
- Consent Form, V2
- Patient Information Sheet, V2
- Generic Data Collection, V2
- Localised data collection sheet, V2
- Inclusion / exclusion criteria, V2

Whole Body (Large Burns) Study
- Standardised Protocol large burns (whole body BIS), V2
- Consent Form, V2
- Patient Information Sheet, V2
- Generic Data Collection, V2
- Whole body data collection sheet, V2
- Inclusion / exclusion criteria, V2 2

The HREC approval is **valid to 04/04/2017** and on the basis of compliance with the 'Conditions of HREC Approval for a Research Project' (attached).

This letter constitutes ethical approval only. This project cannot proceed at the news site/s until separate authorisation has been obtained from the Chief Executive, or delegate, of the site/s under whose auspices the research will be conducted.

To obtain approval at the new site/s a copy of this ethical approval letter must be submitted by the site Principal Investigators to the Research Governance Office at each participating institution as part of the **Site Specific Assessment (SSA)** process.

The RPH Ethics Committee is registered with the Australian Health Ethics Committee and operates according to the NHMRC National Statement on Ethical Conduct in Human Research and International Conference on Harmonisation – Good Clinical Practice.

Should you have any queries about the HREC approval please contact (08) 9224 2292. The HREC's Terms of Reference, Standard Operating Procedures, membership and standard forms are available from the Ethics Office, (08) 9224 2292 or rph.hrec@health.wa.gov.au.

Yours sincerely

PROF FRANK VAN BOCKXMEER
Chairman, Royal Perth Hospital Ethics Committee

CONDITIONS OF HREC APPROVAL FOR A RESEARCH PROJECT

The following general conditions apply to the research project approved by the Human Research Ethics Committee (HREC) and acceptance of the approval will be deemed to be an acceptance of these conditions by all investigators involved in the research project:

1. The responsibility for the conduct of the projects lies with the Coordinating Principal Investigator (CPI). All correspondence with the Lead HREC should be signed by the CPI.

2. Projects that do not commence within 12 months of the approval date may have their approval withdrawn. The CPI must outline why the project approval should stand.

3. The submission of an application for HREC approval will be deemed to indicate that the investigator/s and any sponsor recognises the approving HREC is registered with the National Health and Medical Research Council (NHMRC) and that it complies in all respects with the National Statement on Ethical Conduct in Human Research and all other national and international ethical requirements. **The HREC will not enter into further correspondence on this point.**

4. A list of attendance at a specific HREC meeting is available on request, but no voting records will be provided.

5. The CPI will notify the HREC of his or her inability to continue as CPI and will provide the name and contact information of their replacement. Failure to notify the HREC may result in the project being suspended or approval withdrawn.

6. The CPI will notify the HREC of any departures of named investigators. The CPI will also notify the HREC if any new investigators and/or sites join the project that will utilise the HREC's approval.

7. The CPI will inform the HREC about any changes to the project. The CPI is responsible for submitting any amendments to the approved documents listed on the approval letter, or any new documentation to be used in the project. Any new or amended documentation should be submitted in a timely manner and cannot be implemented at any participating site until they have received HREC approval.

8. The CPI is responsible for reporting adverse events, indicating whether or not the project should continue. Reporting requirements are as per the WA Health Research Governance and Single Ethical Review Standard Operating Procedures. Additional reports other than those outlined that are submitted to the HREC will be returned without acknowledgement. The HREC can request additional reporting requirements as a special condition of a research project.

9. Where a project requires a Data Safety Monitoring Board (DSMB) it is the CPI's responsibility to ensure this is in place before the commencement of the project and the HREC notified of this. All relevant reports from the DSMB should be submitted to HREC.

10. For projects where the site is acting as the sponsor (ie. investigator initiated project) it is the responsibility of the CPI to report serious and unexpected drug/device reactions, as well as other reactions/events to the Therapeutic Goods Administration (TGA). Please refer to TGA website for further information and the relevant forms (see http://www.tga.gov.au/pdf/clinical-trials-guidelines.pdf p71 for medications or p77 for devices).

11. If this project involves the use of an implantable device a properly monitored and up to date system for tracking participants is to be maintained for the life of the device in accordance with the National Statement section 3.3.22 (g).

12. The investigator is responsible for notifying the Therapeutic Drugs Administration of a device incident in accordance with the National Statement section 3.3.22 (g).

13. An annual report on an approved research project will be required on the anniversary date of the project's approval. HREC approvals are subject to the submission of these reports and approval may be suspended if the report is not submitted.

14. The HREC has the authority to audit the conduct of any project without notice. Exercise of this authority will only be considered if there are grounds to believe that some irregularity has occurred, if a complaint is received from a third party or the HREC decides to undertake an audit for Quality Improvement purposes.

15. The HREC can conduct random monitoring of any project. The CPI will be notified if their project has been selected. The CPI will be given a copy of the monitor's report along with the HREC and Research Governance Office (RGO) at each site.

16. Complaints relating to the conduct of a project should be directed to the HREC Chair and will be promptly investigated according to the Committee's complaints procedures.

17. CPI are reminded that records of consent or authorisation for participation in a project form part of the Acute Hospital Patient Record and should be stored with that record in accordance with the WA *Health Patient Information Retention and Disposal Schedule (Version 2) 2000.* A copy of the 'Participant Information Sheet' should also be included in the medical records as part of informed consent documentation.

18. The duration of HREC approval for a project is 3 year (with the option of 5 years) from the date of approval. The date of approval expiry is stipulated in the HREC approval letter.

19. If the project is to continue beyond the stipulated approval expiry date a request for an extension should be submitted prior to that expiry date. One extension of 3 years can be granted but approval beyond this time period may necessitate further review by the HREC.

20. Once the approval period has expired, the CPI is required to submit a final report. If the report is not received within 30 days the project will be closed and archived. An outstanding final report could impact on the CPI's ability to apply for approval for future projects.

21. If a project is suspended or terminated by the CPI, or a project sponsor, the CPI must immediately inform the HREC and the RGO at each site of this and the circumstances necessitating the suspension or termination of the project. Such notification should include information as to what procedures are in place to safeguard participants.

22. If a project fails to meet these conditions the HREC will contact the investigator(s) to request they rectify the identified issues. If, after being contacted by the HREC, the issues are not addressed the HREC approval will be withdrawn. The HREC will notify the RGO at each site within WA Health that work may no longer be conducted in relation to the project other than that concerning the participants safety.

Appendix M NDU Ethics Approval

THE UNIVERSITY OF NOTRE DAME AUSTRALIA

19 Mouat Street (PO Box 1225)
Fremantle, Western Australia 6959
Telephone: +61 8 9433 0555
Facsimile: +61 8 9433 0544
Email: enquiries@nd.edu.au
Internet: www.nd.edu.au

ABN: 69 330 643 210
CRICOS PROVIDER CODE: 01032F

7 October 2014

Dr Will Gibson & Ms Pippa Kenworthy
School of Physiotherapy
The University of Notre Dame, Australia
Fremantle Campus

Dear Will and Pippa,

Reference Number: 014139F

Project title: "Clinical translation of bioimpedance spectroscopy for monitoring the impact of interventions for burn swelling."

Your response to the conditions imposed by the university's Human Research Ethics Committee, has been reviewed and based on the information provided has been assessed as meeting all the requirements as mentioned in the *National Statement on Ethical Conduct in Human Research* (2007). Therefore, I am pleased to advise that ethical clearance has been granted for this proposed study.

All research projects are approved subject to standard conditions of approval. Please read the attached document for details of these conditions.

On behalf of the Human Research Ethics Committee, I wish you well with your study.

Yours sincerely,

Dr Natalie Giles
Research Ethics Officer
Research Office

cc: Prof Peter Hamer, Dean, School of Physiotherapy;
A/Prof Shane Patman, SRC Chair, School of Physiotherapy.