The Biomechanics of Negative-Pressure Wound Therapy

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"Men love to wonder, and that is the seed of science."

Ralph Waldo Emerson

This work has been financially supported by the Department of Surgery, University of Cape Town, the Faculty of Health Sciences, University of Cape Town and the Martin Singer Hand Unit, Department of Orthopaedic Surgery, University of Cape Town, South Africa. The NPWT dressings for some studies were donated by Kinetic Concepts, Inc., South Africa.

The experimental work was carried out at Groote Schuur Hospital, Cape Town and in part at the University of KwaZulu-Natal, Durban, South Africa.

For Mel and my ten-week-old little princess, Grace

and for my mother, Pitsa and late father, Demetri Kairinos

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Abstract

Introduction

Despite the success of negative-pressure wound therapy, its mechanism of action remains unclear. The common perception that it reduces tissue pressure and increases perfusion has recently been challenged following the observation that tissue necrosis can be caused as a result of its application.

A programme of research has been conducted to clarify how tissue pressure changes during negative-pressure wound therapy and the resultant effect thereof on perfusion. The cause for conflicting evidence from other studies was also investigated.

Materials and Methods

Using a strain-gauge transducer, both *in vitro* and *in vivo* tests were done to assess tissue pressure changes as a result of different configurations of negative-pressure wound therapy dressings (circumferential, non-circumferential and dressings in a cavity). Perfusion changes were analysed using radioisotope perfusion imaging, transcutaneous partial pressure of oxygen monitoring and thermography.

To investigate the cause for conflicting evidence in the literature, a hypothesis was tested regarding a potential flaw of the laser Doppler device, which is the most commonly used modality for measuring perfusion in this field. Additionally, a study was undertaken to assess the influence that manufacturers have on the outcomes of comparative scientific research studies on negative-pressure wound therapy.

Results

All configurations of negative-pressure wound therapy were found to increase tissue pressure. Perfusion was found to be reduced in the two dressing configurations tested (circumferential and non-circumferential).

The findings of the laser Doppler study supported the hypothesis that this modality's method of measuring perfusion is flawed in the setting of negative-pressure wound therapy. The involvement of a manufacturer in a study comparing the two commonly used forms of negative-pressure wound therapy correlated to scientific outcomes that favoured that specific manufacturer's type of negative-pressure wound therapy dressing.

Conclusion

This research suggests that tissue pressure increases and perfusion reduces as an immediate consequence of negative-pressure wound therapy, contrary to common perception.

Previous data on perfusion which conflicts with this finding appear to have been confounded by a flaw in the measuring technique of the laser Doppler. Research may be further confounded by the influence of manufacturers on the outcomes of scientific studies.

1 Introduction

Outline

1.1. Introduction

The treatment of wounds is as old as mankind itself[1], therefore it is quite understandable that the subject of wound healing and dressings/devices to facilitate wound healing, is one of the oldest and largest in the field of medicine. As such, an in-depth review of all the available literature would be impossible and difficult to digest in a literature review; such a review would also be unnecessary to grasp the fundamentals to be discussed in this research project. Therefore, only the literature relevant to the understanding of the research presented herein will be discussed.

The focus of this research pertains to the mechanism of action of negative-pressure wound therapy (NPWT) and more specifically, the effects it has on tissue pressure and perfusion. To a degree, our limited understanding of wound healing has handicapped our quest to understand the mechanism of action of NPWT. However, in this chapter it will be demonstrated that our knowledge of the mechanism of action is hampered even further by the confounding results of numerous basic research studies. Potential methodological inadequacies of these studies will be highlighted, offering likely explanations for their conflicting findings, as well as opportunities for further research. Indeed, many of these opportunities prompted the research presented in this thesis.

1.1.1. Wound healing

Tissue injury is typically followed by a cascade of wound repair processes that usually result in the restoration of tissue integrity. The repair cascade consists of the highly coordinated inflammatory, proliferative, and remodelling phases, usually with considerable overlap between these phases.[2] Except for what is known at a descriptive level, the highly complex process of wound healing remains poorly understood by both scientists and clinicians alike.[3] Although wound healing is a fundamental process occurring in almost all injured tissues, the wounds referred to by most texts (including this) are those of the skin and subcutaneous tissue.[2, 3]

An acute wound is typically defined as one that has occurred within the past three to four weeks.[3] The definition of a chronic wound varies in the literature, ranging from one that has not healed (re-epithelialised) within four weeks[2,3] to one that has not healed after three months.[4] Some advocate de-emphasising chronicity when defining chronic wounds and rather focusing on whether the wound is continuing satisfactorily through the relevant phases of wound healing.[4]

1.1.2. Complex wounds

There are numerous factors that increase the complexity of the wound and include the size of the defect, injury to associated underlying structures/tissue, iatrogenic interventions/medications or comorbid illnesses that influence wound healing, and inability to progress from one phase of wound healing to the next. No formal definition of a "complex wound" exists[5] but a wound that is influenced by the aforementioned factors would typically be called a "complex wound". Complex wounds can therefore be either acute or chronic.

Physicians are presented with an ever-increasing number of these challenging wounds. The increasing proportion of the aging population, obesity, and a concomitant increase in type-2 diabetes are likely contributing factors.[6-8] Increasingly complex operations, radiotherapy, immunosuppresion, complex war injuries, infections from resistant bacteria and an increasing number of pressure sores have further aggravated this problem, making the care of wounds one of the most significant challenges for healthcare systems today.[7] Wound-care, particularly advanced wound-care, has therefore become increasingly important in the field of medicine.

1.1.3. Brief history of wound-care

Although wounds were dealt with as far back as the caveman era,[9] the first records containing written medical information regarding wound-care can be found in the papyri from Egypt, dating approximately 3100 BC.[1] Wound-care has undergone a

considerable metamorphosis from that era to the present time. Initial knowledge of wound-care was derived through trial and error and often shrouded in mystery, magic and customary beliefs. Practically every substance imaginable was tried, ranging from herbs to powdered snails, animal fat to animal dung,[1] some with better outcomes than others. Our present insight into wound healing is considerably more scientific. Our understanding of the purpose of inflammation only came about after 1908, when Ilya Metchnikoff won the Nobel Prize for his discovery of phagocytosis.[10] Following that, the last century has been characterised by many significant discoveries in the wound-healing process, including the role of cytokines, growth factors and stem cells, to name but a few.

The earliest forms of bandages were the leaves or grasses often used to soothe wounds.[1] In 2500 BC, the Mesopotamians used bandages which may have been made of wool or linen.[1] By the fifth century BC, surgeons knew that bandaging too tightly could cause gangrene.[1] In 1891 Johnson & Johnson was the first company to mass-produce sterile surgical dressings.[11] Various advances on the gauze dressing have been made over time, each designed to fit specific wound requirements. For example, dressings with different degrees of absorption have been used for different amounts of exudate. Some dressings are antibacterial, while others are anti-inflammatory. Some dressings have been used to debride necrotic tissue, either by hydration or with the use of chemical agents. Skin substitutes have also enjoyed particular attention in recent years, although it must be said that they are a form of definitive treatment, rather than a temporary dressing. Manufacturers often claim that their dressing is ideal for all types of wounds. However, the existence of a dressing suited to all wound circumstances needs to be questioned as no wound is the same. Despite the increased cost of these advanced wound dressings, they have enjoyed increasing popularity over the traditional gauze-based dressings.[12]

The advanced wound-care market has also been compounded by the introduction of an increasing number of wound-care devices, for example, ultrasound therapy, phototherapy, electrostimulation, nanodelivery of targeted therapy, and NPWT.[12] Presently, the size of the advanced wound-care market has already superseded that of the traditional wound-care market and, in a 2005 report, was forecast to be about $3 billion by 2011.[12] The increasing success of NPWT has assisted with this rapid expansion.[12]

1.2. Negative-pressure Wound Therapy (NPWT)

1.2.1. Concept of NPWT

NPWT, as we know it today, comprises an interface dressing (foam or gauze) being applied to a wound, with or without a non-adherent contact layer between the interface dressing and the wound. This is then covered by an adhesive occlusive drape to create an airtight seal around the wound. Some form of suction tubing is then placed into or over the interface dressing by cutting a hole in the adhesive occlusive drape. When the hole is sealed off with more adhesive occlusive drape or by any other means, one can apply suction to the tube, which is in contact with the interface dressing. Applying suction will evacuate air from the dressing and create a hypobaric pressure within the dressing (often referred to as "negative-pressure"). Numerous iterations of the above-mentioned technique exist and will be described in further detail later.

1.2.2. History of NPWT

NPWT has been one of the most significant advances in wound-care in the last century, as demonstrated by the fact that the second and third most frequently cited publications in plastic surgery literature are both studies on NPWT.[13] These two landmark studies, by Argenta *et al.*[14] and Morykwas *et al.*,[15] were published back-to-back and focused on the clinical experience and also the basic scientific

foundation on which NPWT is based. Argenta and Morykwas were also co-inventors of the Vacuum Assisted Closure (V.A.C., Kinetic Concepts Inc., San Antonio, TX, USA) device, which has dominated the NPWT market since it was popularised by their publications in 1997. Although these publications heralded the start of the NPWT era, numerous less well-known papers pre-dated these reports.

Perhaps the first mention of a form of negative-pressure therapy was in 280 BC, with the invention of the syringe by a Greek barber.[9] This was used for, among other things, sucking pus out of wounds. Paul Svedman, a plastic surgeon, was an innovator in wound irrigation and combined this with suction. He published the first such article in 1979.[16] The product used displayed many similarities to current examples of NPWT in that it comprised an interface dressing and suction tubing (to remove the irrigation fluid), which was covered by an adhesive occlusive drape. Miller *et al.* quote a series of publications derived from the Russian literature that described the treatment of wounds using negative-pressure.[17-20] These became known colloquially as the "Kremlin papers"[21] and are discussed in further detail in various components of this chapter. Numerous other papers on vacuum therapy were also published by these and other Russian authors.[22-30] The "Kremlin papers" concluded that treatment of wounds using negative-pressure was beneficial. Among other benefits, the following were cited to occur in wounds treated with negative-pressure: a reduction in bacteria,[17, 18, 20] faster wound healing and less cicatrisation.[18] These papers will be discussed in further detail in the subsection, "Tissue pressure and the biomechanical effects of NPWT".

In addition to the Russian papers, there was also considerable work on NPWT published in the German literature by Fleischmann *et al.*[31-34] Good results were reported on wound healing and tissue infection, using this modality. A more well-known paper than the aforementioned papers, which was also published prior to Morykwas' and Argenta's key papers, is that of Chariker *et al.*[35] Mark Chariker

was a resident when his paper was published in 1989, describing a "closed suction wound drainage system", which was used on seven patients between 1984 and 1986. Their system comprised gauze as an interface dressing and a Jackson-Pratt suction drain; these were then sealed off with transparent adhesive film. They reported good results when managing incisional and enterocutaneous fistulae, with all fistulae closed within a mean time of 16 days.

There are currently over a thousand articles on NPWT.[36] However, despite the obvious success of NPWT, the number of well-designed scientific or clinical studies which support its efficacy are lacking.[6, 36-39] Nevertheless, the continued success of NPWT has resulted in some recommending that it forms part of the reconstructive ladder.[40]

Despite the numerous patents filed by Kinetic Concepts Inc. (K.C.I.), other manufacturers have also entered the market with similar devices, albeit with minor variations. For example, most of these companies have avoided using the open-cell foam that K.C.I. uses due to potential patent infringements. Instead, they have used gauze-based interface dressings in place of the foam, which were popularised by Chariker *et al.* in 1989.[35, 41] It is this "Chariker-Jeter" system that K.C.I.'s largest competitor, Smith and Nephew, uses. Although, there are numerous smaller companies that have joined the NPWT market, these two larger rivals have been locked in patent infringement battles for numerous years. This has instigated numerous articles comparing foam-based and gauze-based dressings.[42-52] However, the level of evidence to support a particular dressing interface remains poor. Interestingly, the majority of studies that have demonstrated equal or greater benefits when using the Chariker-Jeter technique (gauze interface, with perforated drainage tube supplying lower suction pressures),[42-46, 48, 53-56] appear to be funded or assisted by, or have co-authors employed by, companies that use the Chariker-Jeter technique (every company, other than K.C.I.). Similarly, studies that demonstrate greater benefits from the use of foam,

K.C.I.-patented drainage systems or the higher suction pressures advocated by K.C.I. appear to be funded by, or have disclosures (paid consultant, employee, etc.) relating to K.C.I.[6-8, 47, 49, 50]

1.2.3. Overview of the mechanism of action of NPWT

Despite this therapy's rapid introduction into clinical practice, the mechanism by which it stimulates wound healing continues to elude us.[6, 48, 54-63] Many theories have been put forward to explain the success of these dressings.[6, 7, 14, 15, 49, 58, 64-68] The majority of these theories are promulgations of those put forward in the seminal work of Morykwas and Argenta *et al.*,[14, 15] where the following beneficial factors were demonstrated: increased local perfusion, accelerated granulation tissue formation, decrease in bacteria and an increase in nutrient flow to random pattern skin flaps (as determined by increased flap survival). Further studies have demonstrated a reduction in wound surface area,[69-73] regulation of inflammatory cytokines,[74-76] and other proteins.[50, 77] Reduced pCO_2 and increased pO_2 and lactate were observed in wound fluid, while pH and bicarbonate remained unchanged.[60] The reasons for the above-mentioned occurrences are postulated to include a reduction of oedema, an increase in local blood flow, micro- and macrodeformational forces on the wounds, and reduced heat loss.[6, 14, 15]

This review demonstrates that there are numerous studies that conflict with one another and it is the author's contention that this is partly due to an incomplete understanding of the biomechanics of these advanced dressings. An improved insight into the physics and biomechanics of NPWT (gained during the author's research) has resulted in the identification of potential flaws in the methodologies of many of these studies. This has allowed for potential explanations for the conflicting findings. The relevant literature and potential flaws will be discussed under two headings, namely, "Tissue Pressure and biomechanical effects of NPWT" (section 1.3.) and "NPWT and perfusion" (section 1.4). Various topics relevant to these headings will be discussed under appropriate subheadings.

1.3. Tissue pressure and the biomechanical effects of NPWT

1.3.1. The physiological response of tissues to hypobaric pressure

The interstitial fluid hydrostatic pressure of normal tissue is slightly hypobaric (-1 to -2 mmHg)[78-82] and it has been shown that trauma, particularly burns, results in this becoming even more hypobaric.[81, 82] This increased hypobaric interstitial pressure is likely to be the cause of fluid being drawn into the interstitium (oedema) when vessels develop increased permeability due to inflammatory mediators.[81, 82]

It has been stated that the application of any form of positive pressure leads to a decrease in skin perfusion and hypoxia, while negative pressure increases skin perfusion.[83] However, in contrast to this, it has also been demonstrated that negative pressure applied to tissues results in vasoconstriction,[84] serving as an oedema-protective factor.

1.3.2. Tissue pressure beneath NPWT

It would seem intuitive that a medical device that has revolutionised wound-care using hypobaric pressure would have resulted in numerous studies on the effect this device has on tissue pressure and the physiological ramifications thereof. Yet few studies have investigated the tissue pressure changes beneath a NPWT dressing.[85-87] This is likely to be due to the fact that, to most minds, a device that creates hypobaric pressure over a wound is likely to reduce tissue pressure.[88-92] *If* this does occur, one would question whether it occurs due to the suction force (immediate) or whether it occurs as a secondary effect after a reduction of oedema (which would occur over some time). An even more intriguing question is whether tissue pressures are reduced at all – or perhaps even increased (although this seems counterintuitive). When reviewing studies that have investigated this, one must distinguish between *tissue pressure* and *air pressure at the wound surface;* these pressures may not be equivalent or even similar.

Two of the three articles investigating tissue pressure beneath NPWT are from the German literature.[86] These researchers found that tissue pressure is increased when exposed to NPWT *in vitro* and also in human wounds. The study by Maier *et al.*, mentioned that "only a slight positive external pressure" of 31 mmHg occurs at a suction pressure of -40 kPa (-300 mmHg) on the wound surface.[86] Although this may be seen as "slight", the implications thereof may be significant when one considers the fact that the average capillary perfusion pressure ranges between 10 and 30 mmHg.[79]

In the English literature, Murphy *et al.* used a pressure transducer to investigate the depth of penetration of negative pressure into the tissues beneath a NPWT foam dressing (on rabbit wounds).[87] In contrast to the previous two studies, they found that NPWT creates negative interstitial pressures, and that the higher the suction pressure the deeper the depth of penetration of these hypobaric pressures. At the maximum suction they used (-200 mmHg), the depth of penetration did not penetrate to more than 1 mm below the surface of the wound. This study was subsequently challenged because of a likely flaw in their methodology,[93] as their transducers were placed through the foam dressings and into the underlying tissues. It was proposed that this technique was unlikely to measure the true interstitial fluid pressure because the tunnel created in the tissues through which the transducer was placed, was in continuity with hypobaric pressure within the foam of the NPWT dressing. This could have resulted in spurious hypobaric pressure readings, which may not have been a true reflection of the interstitial fluid pressures. A different reading may have been obtained if the authors had eliminated the exposure of the transducer's tunnel to the hypobaric pressure within the dressing. This could have been achieved by placing the entry site of the tunnel away from the NPWT dressing. This may explain why their findings conflict with those of the aforementioned two German studies,[85, 86] where it was found that NPWT created hyperbaric tissue pressures.

From this small volume of literature, it appears more likely that the immediate effect of NPWT is to increase tissue pressure,[86] contrary to common perception.[88-92] However, due to the small number of studies and the conflicting results, there is currently a poor understanding of how NPWT affects tissue pressure. It should be noted that the previously discussed studies explored only the immediate effects of NPWT on tissue pressure and there are no studies that have explored the effects of NPWT on tissue pressure over a prolonged period of time.

1.3.3. Pressure-transducing capabilities of interface dressings

There are considerable conflicting findings relating to whether the type of interface dressing influences the pressure reaching the wound surface. Again, it must be stressed that one must differentiate the concept of pressure created at the wound surface and pressure created within the tissues, as these are not necessarily similar. Willy *et al.* compared the capability of polyvinyl alcohol (PVA) foam and polyurethane (PU) foam to transduce negative pressure through the material.[85] This was done by measuring the pressure in the foam at varying distances (in the horizontal plane) from the mouth of the suction tube. It was found that PVA foam is less capable of transmitting negative-pressure than PU foam, indicating that the type of interface dressing used does influence the amount of negative-pressure transmitted to the wound surface.[85] At suction pressures of -125 mmHg, PU foam transmitted almost 100% of the hypobaric pressure up to 60 cm from the source, while PVA foam demonstrated a reduction of up to 25% at a distance of 15 cm.[85]

Malmsjo *et al.* compared the capabilities of foam and gauze to transduce negative pressure to the wound.[46] It was found that there was no significant difference between the two. A near-linear, directly proportional increase in negative pressure occurred at the wound surface for both interface dressings.[46] As with the previous study, it must be borne it mind that the "negative pressure" referred to in this paper is not necessarily the same as those within the tissues. These investigators

used a saline-filled catheter, which was connected to a pressure transducer and sutured to the wound surface.

Although both of these conflicting studies attempted to enhance our understanding of how interface dressings affect the transmission of hypobaric pressure to the wound, their findings are not comparable. Malmsjo *et al.* compared PU foam interfaces to gauze interfaces, while Willy *et al.* compared PU foam to PVA foam. Malmsjo *et al.* used a saline-filled catheter to measure pressure, while Willy *et al.* used a strain-gauge piezoelectric pressure transducer. Most important, however, is the fact that Malmsjo *et al.* were measuring vertical pressure transduction, while Willy *et al.* were measuring horizontal pressure transduction. Therefore, the distance from the mouth of the suction tube to the pressure sensor was much shorter in Malmsjo's experiments than Willy's. It may be possible that Malmsjo's experiments were therefore less likely to demonstrate a difference in the pressure-transducing capabilities of the two materials being tested, as there was considerably less material between the suction tube's opening and the sensor.

Yet another confounding factor in the study by Malmsjo *et al.* is that they used a different dressing application technique for the two interface dressings, adding an additional variable.[46] The manufacturer's T.R.A.C. (Therapeutic Regulated Accurate Care) pad was used for suction delivery to the foam dressing, as is recommended when using the V.A.C. technique.[94] This is always placed *on top* of the foam. However, a commercially available Jackson-Pratt drain was used with the gauze dressing, which is always placed *within* the gauze, as in the Chariker-Jeter technique.[35] This is likely to position the suction delivery closer to the pressure sensor (at the base of the wound) in the gauze than the foam. Although Malmsjo *et al.* concluded that there was no difference in the ability of foam or gauze to transduce pressure to the bottom of the wound,[46] this cannot be deduced from their study. Their findings merely indicate that, in the shallow wound created in that particular experiment, a similar pressure reaches the wound when

using either of the two techniques (V.A.C. or Chariker-Jeter).

Subsequent to this, Malmsjo *et al.* undertook a similar study – this time on a larger, deeper (porcine) wound, measuring both horizontal and vertical pressure transduction, in wet and dry dressings. Both types of suction-delivery tubes (T.R.A.C. pad or perforated drains) were also tested on either interface dressings. Again, it was concluded that there was no difference in the pressure-transducing capabilities of foam or gauze, regardless of suction source.[46] It was, however, demonstrated that when either of the two interface dressings was wet, the ones that made use of a T.R.A.C. pad had a significant pressure drop-off at the wound surface compared to those that had perforated drains.[46]

In contrast to Malmsjo's work, McNulty *et al.* subsequently found significant differences in the horizontal and vertical pressure-transducing capabilities of gauze and foam.[49] Like Willy *et al.*,[85] they used a strain-gauge pressure sensor to measure pressure in the dressing.[49] This study conducted the experiments using the T.R.A.C. pad in both types of interface dressings and also the Jackson-Pratt drain in both, thereby eliminating the suction system as a variable. An additional component of this study demonstrated that the column of wound fluid in the suction tube can affect the pressure delivered to the wound, particularly when the suction pump is higher than the wound.[49] The patented design of the T.R.A.C. pad allows the pump to sense if the pressure at the pad is different to that prescribed at the pump. If a pressure drop has occurred at the T.R.A.C. pad, the pump increases the degree of suction, thereby ensuring that the prescribed suction pressure reaches the pad, regardless of fluids in the tube or the height of the pump.[49] This fact is important when analysing studies that use different manufacturers' systems to compare the effects of gauze and foam at a given suction pressure; the device that does not make use of a K.C.I. pump with the T.R.A.C. pad may be generating different pressures at the interface dressing, thereby confounding the results.

Interestingly, if the T.R.A.C. pad is designed to overcome pressure drop-off, one may ask why Malmsjo *et al.* found that the T.R.A.C. pad resulted in a significantly greater pressure drop-off (when used on wet dressings) than the perforated drainage tubes.[46] Although we can only speculate, it may be that the suction pump used was not a K.C.I. pump, which is necessary for the T.R.A.C. pad to be effective; it has the software to increase the degree of suction when a pressure drop is sensed within the T.R.A.C. pad. Malmsjo *et al.* did not mention what type of pump they used as a suction source.[46] However, a Smith & Nephew employee was involved in the study and the study was partly funded by this manufacturer, making it possible that a Smith & Nephew pump was used, rather than the K.C.I. pump.

In an attempt to investigate the level of negative-pressure transduction in the thoracic cavity when NPWT is applied to a sternotomy wound, Tobrand *et al.* used saline-filled catheters, placed at various locations in the chest cavity, at varying distances from the foam.[90] One was placed beneath the foam (between the foam and the heart), another was placed behind the heart in the pericardium, a third was placed in the oesophagus via the mouth and yet another was placed in the pleura. These investigators demonstrated that although negative-pressure increased in a linear fashion beneath the foam on the anterior surface of the heart, there was very little change in pressures in the deeper locations.[90] They concluded that the reduced pressure was only found in the immediate proximity to the foam. According to these investigators, this explained the findings of previous studies, by Wackenfors *et al.* that demonstrated increased perfusion at the wound edge, while tissue further from the vacuum source remained unaffected.[59,60] However, Wackenfors' studies did not demonstrate an increase in perfusion at the wound edge, but rather a reduction in perfusion[60] (discussed later). Therefore, rather than explaining Wackenfors' findings, Tobrand's findings (that there was a reduced pressure in the immediate proximity to the foam) conflict with them.

1.3.4. Pressure-transducing capabilities of non-adherent contact dressings

Occasionally non-adherent dressings, such as paraffin gauze, are placed between the interface dressing and the wound. These serve to minimise the wound surface sticking to the interface dressing. This is particularly relevant to PU foam interface dressings, where granulation tissue tends to grow into the foam interstices.[15, 48, 95] These dressings are also recommended when NPWT is used over organs (heart, intestines) or exposed tendons or vessels.[94] These dressings have been found to reduce the amount of negative pressure transferred to the wound surface, which could influence the mechanism of action of the NPWT dressing.[90, 95] These findings, however, are not supported by Malmsjo *et al.* who could not demonstrate a diminution in the negative pressure transferred to the wound surface.[43] They proposed that the likely reason for their findings not echoing those of others was the fact that the wounds in their experiments were shallow.[43] An additional reason for this may be that the non-adherent dressing used by Malmsjo *et al.* was not the conventional paraffin gauze dressing (Jelonet, Smith and Nephew, Hull, UK), which is covered with a thick layer of paraffin, which could block the suction delivery channels within the interface dressing. Jones *et al.* demonstrated that the type of non-adherent contact dressing affected the degree of pressure delivery to the wound, with paraffin gauze resulting in the greatest pressure change.[95]

1.3.5. Macrodeformation

The immediate pressure changes demonstrated in tissues undergoing NPWT[85, 86] have not yet been explained.[6, 8] There is a paucity of literature investigating whether they are due directly to the hypobaric pressure above the wound or due to the macrodeformation of the tissues that occurs as a result of the interface dressing contracting to a smaller volume. "Macrodeformation" refers to the strain created by the negative-pressure dressing on the walls of the wound, usually resulting in an alteration in the shape of the wound as the dressing contracts. Malmsjo *et al.* compared the deformational forces that gauze and foam interface dressings exert on the wound edge in a porcine wound model.[43] There was no difference

in the amount of macroscopic deformation that occurred in wounds created on the back of a pig.[43] However, a subsequent study on sternotomy wounds by the same author, demonstrated that foam exerts a greater deformational force than gauze does.[45] The authors attributed this discrepancy to the different locations of the wounds in each study, with the stiff skin on the pig's back allowing little deformation,[43] while the more mobile sternal edges allowed for considerable movement.[45] In the same article, it was also demonstrated that gauze offers more resistance to distraction forces, conferring more protection to mobile sternal edges.[45] Gauze also appeared to result in less trauma to underlying cardiac and lung tissue than foam.[45] Other *in vitro*[49] and *in vivo*[96] studies have confirmed that PU foam results in significantly greater deformation than does gauze.

1.3.6. Microdeformation

Microdeformation is similar to macrodeformation, although this type of deformation occurs at a microscopic level, culminating in the typical undulations seen on the surface of any tissue that has been exposed to NPWT. Although the forces responsible for this can be a combination of compression, sheer and strain, surgeons typically refer to them as "strain".[6, 8, 47, 49, 64, 97] These forces are usually explained in the context of PU foam, whereby it is envisaged that the tissue beneath the pores of the foam is sucked upwards, while the "struts" of the foam around each pore push into the tissues, creating strain on the cells.[47-49, 53, 64, 85, 97, 98] It is well-documented that the application of mechanical forces to cells results in a host of synthetic activities, including production of extracellular matrix components (proteoglycans, glycosaminoglycans, collagen and elastin)[49,99-101] and angiogenesis.[49, 75, 98, 100] In addition, numerous biochemical markers and signalling factors can be down- or up-regulated.[49, 65, 76, 101] Knowledge of the above-mentioned occurrences has provided another likely mechanism of action of NPWT.[15, 47, 49, 64, 65, 97, 102]

Investigating the microdeformational effects of NPWT is challenging when the

interface dressing is still in place while suction is applied.[47, 97] Some studies have inferred that microdeformation occurs based on the histological appearance of the wound surface (when the interface dressing is removed).[6, 7] Although these findings are convincing, there is no confirmation that the undulations observed on a histological specimen are similar to that which would be observed if the interface dressing were still in place with suction applied. Furthermore, during preparation of the tissue for histological evaluation, there can be changes in the morphological appearance of the tissue surface, which could confound the results.

Complicated computer algorithms, using finite element modelling, have been created to predict the three-dimensional strains that tissues undergo during NPWT.[7, 47, 64, 97] Although these studies also demonstrate that microdeformation is likely to occur, they are based on certain assumptions, which do not apply to the conditions of a wound. For example, in the two-dimensional computational model of Saxena *et al.*[64] and also that of Scherer *et al.*[7] it was assumed that the centre of the pore of the foam interface underwent no lateral displacement and that there was no vertical displacement where the wound contacts the dressing. Yet, from our understanding of macrodeformation, it can be envisaged that these conditions are unlikely to occur in a wound. The computational model of Scherer *et al.* also assumes that the adhesive occlusive drape and the foam contact does not result in tissue strain or displacement.[7] Again, with our knowledge of the forces of macrodeformation, these assumptions appear unlikely.

In order to gain an even more accurate understanding of microdeformation, Wilkes *et al.* attempted to overcome many of the assumptions from previous studies by using three-dimensional finite element modelling (FEM) and corroborating these mathematical findings with "real-world conditions" using a custom-made micro-CT test cell.[97] Despite the support this study lent to the previous studies, it too made assumptions that are unlikely to be present in a wound. For example, in

the FEM it was assumed that the adhesive occlusive drape behaves like a rigid plate.[97] However, it is common knowledge that the polyurethane drapes used are pliable, elastic and conform (resulting in wrinkling of the drape) to the underlying foam. In fact, in their diagram of the micro-CT device, the drape can be seen conforming and wrinkling against the underlying foam (Fig. 1) – this is not the behaviour of a rigid plate, as was assumed in their finite element modelling.

Additionally, the micro-CT cell model used by Wilkes *et al.*[97] was not exactly equivalent to a real wound undergoing NPWT. This cell consisted of a homogenous substance within a container to which a NPWT dressing could be applied.[97] CT scans could then be taken to evaluate the microdeformation that occurred while the dressing was applying suction. In theory, this seems a good model to evaluate NPWT. However, the adhesive occlusive drape was applied to the sides of the rigid container. This differs from the scenario of a NPWT. In the latter, the contraction of the interface dressing (on application of suction) results in the drape pulling the surrounding skin and underlying tissue toward the wound in the horizontal plane (macrodeformation). These alterations of tissue strains surrounding the wound environment will undoubtedly alter the forces and mechanical interactions between the dressing and the underlying tissue. There is no provision for this in the micro-CT cell of Wilkes *et al.* as the adhesive occlusive drape is attached to the rigid sides of the container.[97]

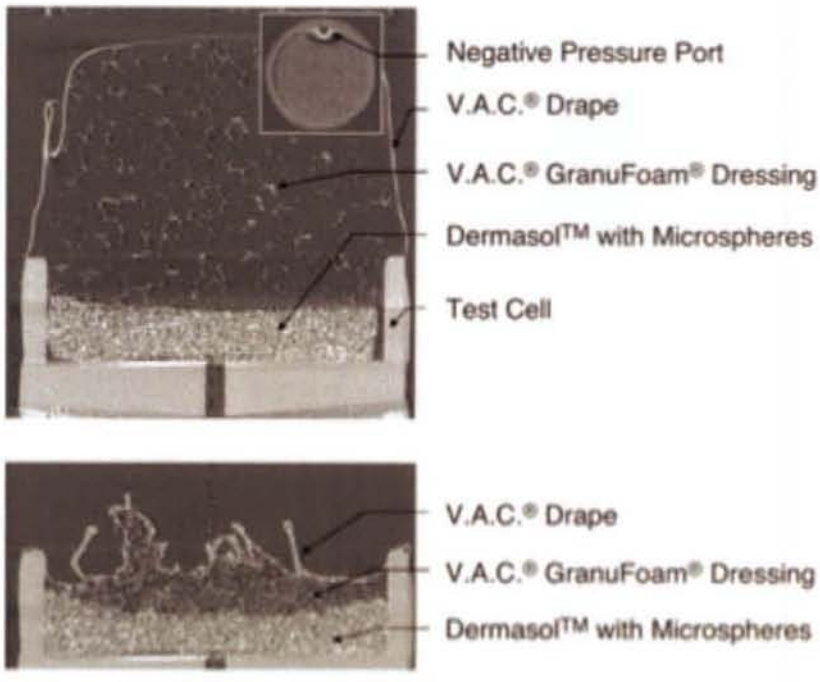

Fig. 1: The micro-CT model created by Wilkes *et al.*[97] Notice the wrinkling of the adhesive drape, which does not act like a rigid plate (as was assumed in their finite element analysis). Additionally, it can be seen that the adhesive drape is attached to the rigid sides of the container. This set up therefore does not allow the occurrence of macrodeformation, which occurs in real wounds. (Reprinted with permission from Wilkes R, Zhao Y, Cunningham K, *et al.* 3D strain measurement in soft tissue: Demonstration of a novel inverse finite element model algorithm on MicroCT images of a tissue phantom exposed to negative-pressure wound therapy. *J Mech Behav Biomed Mater.* 2009;2:272-287.)

1.3.7. Mechanotransduction

Both macrodeformation and microdeformation result in forces which are transferred to the cells within the tissue.[6-8, 47, 64, 97] "Mechanotransduction" refers to the many mechanisms by which these cells convert the mechanical stimuli into chemical activity, including, among others, synthetic activity.[101, 103-105] The sensitivity and resultant response of cells to external forces is as widely researched as it is complicated and falls beyond the remit of this literature review. Simply, the forces applied to the cells from the extracellular matrix are transferred, via transmembrane integrin bridges, to the cytoskeleton.[15, 101, 103, 104] The integrins are sensitive to these

forces and result in the release of second messengers, such as prostaglandins, inositol phosphates, protein kinase C and intracellular calcium.[15] Depending on the duration and magnitude of the force and the cell type, numerous responses can be effected by the cell.[72] Among the effects are re-orientation (either parallel or perpendicular, to a certain force),[72, 104] cell growth and proliferation,[7, 15, 66, 106] cell survival[104, 106] and synthesis of various molecules.[15, 72, 97, 104]

Most *in vitro* studies on mechanotransduction have been conducted in a two-dimensional plane.[97] These experiments are commonly carried out by planting cells on silicone membranes, which are then stretched.[107-111]. Cell-strain apparatuses have been designed,[112-117] although not specifically for NPWT. Wilkes *et al.* designed a "bioreactor" specifically for the purpose of studying the three-dimensional strain experienced by cells during application of NPWT.[102] Their study served only to validate the viability of the device and obtain preliminary data on cellular responses; future work was planned to investigate the effects of NPWT on protein synthesis and cell signalling.[102] However, the bioreactor in the study of Wilkes *et al.* may not be a precise replication of what occurs beneath a NPWT dressing *in vivo*. The reasons proposed are the same as those given regarding their micro-CT cell,[97] discussed earlier.

1.3.8. Histological effects of NPWT in relation to biomechanical forces

NPWT significantly increases the rate of granulation tissue formation, as demonstrated by Morykwas *et al.*[15] Wounds on the backs of pigs demonstrated a mean increase in granulation tissue of 63% for continuous NPWT and 103% for intermittent NPWT, significantly greater than control wounds ($p<0.01$ for both).[15] It has also been demonstrated that NPWT at -125 mmHg (using PU foam) increases the rate of granulation tissue formation more than NPWT at higher or lower pressures ($p<0.0001$).[71] Interestingly, this study also noted that wounds deteriorated significantly ($p<0.0001$) when a deliberate hole was created in the drape, despite a negative pressure of -123 mmHg still being generated.[71] Both

of the aforementioned studies determined granulation tissue volumes using the principle of volume displacement.[15, 71] In order to measure granulation tissue, all the wounds were filled with alginate impression material (Jeltrate; Dentsply International, Inc., Milford, DE). Once the material had set, it was removed and placed in a water-filled graduated container and displacement was measured.[15, 71]

The latter study could contain an element of subjectivity, as wounds were debrided of any "non-viable" tissue every 48 hours, prior to measurement.[71] Any bias could (either intentionally or unintentionally) result in one wound being debrided more than another. Prior research had resulted in two of the authors (Morykwas and Argenta), who are also inventors of the V.A.C., advocating that -125 mmHg is the optimal pressure to use,[14, 15] which was widely publicised during the world-wide commercialisation of the product. It could be argued that if they were to demonstrate more successful effects at another pressure, other than -125 mmHg, it would conflict with their previous research and also the marketing drive. If this were to be the case, this too would add an element of bias to their study.

The effects that NPWT has on the tissue parenchyma can be due to a number of reasons. Simply, it could be due to either the interface dressing itself interacting with the underlying tissue, the effect that the hypobaric pressure has on tissue, the fact that the wound is sealed off by a semi-occlusive dressing, or a combination of these. An important study by Scherer *et al.* deserves some discussion in this regard.[7] An experiment was conducted to dissect these four components, to determine their effects on vascularity and cell proliferation. Five groups were created in a murine model: Group 1 – adhesive occlusive dressing, without interface dressing or suction (OD); Group 2 – adhesive occlusive dressing, without interface dressing but with suction (Suction); Group 3 – PU foam with adhesive occlusive dressing, no suction (Foam); Group 4 – PU foam with adhesive occlusive dressing, no suction, but with a compressive force applied (170g/cm2), which should be equivalent to the pressure of 125 mmHg (Foamc); Group 5 -

conventional V.A.C. with suction of -125 mmHg (V.A.C.). At day 7, measurement of the granulation using microscopy revealed that the Foamc group and the V.A.C. group had significantly more than the rest of the groups; there was no significant difference between the rest of the groups.[7]

Scherer's study also used immunohistochemistry to evaluate cell proliferation (using Ki67, a marker for actively replicating cells) and vascularity (using PECAM1, a panendothelial surface marker).[7] It was demonstrated that cell proliferation was significantly ($p<0.005$) increased only by the composite dressing i.e. Group 5 (foam and suction).[7] From this it was deduced that the composite device was necessary for cell proliferation.

It was further demonstrated that all three dressings containing foam (Group 3-5) resulted in significantly increased vascularity ($p<0.05$) compared to the others, i.e. PU foam (even without suction) increases vessel formation.[7] In fact, the uncompressed foam without suction (Group 3) produced as much vessel formation as did the composite V.A.C. (Group 5).

The group that had suction but no foam (Group 2) had significantly less vessel formation than the foam-containing groups; therefore Scherer *et al.* concluded that *suction forces* alone might not directly be able to stimulate vessel formation.[7] They proposed that the interface material itself (in this case PU foam) may actually have stimulatory effects on vessel formation. This prospect is an important one, as there is on-going debate as to the importance of the type of interface dressing used. Therefore further discussion about the validity of this deduction is warranted.

Scherer's deduction that suction might not be able to stimulate vessel formation[7] is potentially flawed; Group 2, the "suction without foam" group, had little, if any suction applied to the tissues because without an interface dressing acting as a manifold, the adhesive occlusive drape simply collapses under the hypobaric

pressure. This, in all likelihood, prevents any hypobaric air coming into contact with the tissues of the wound. Even if there was some contact between the air and the tissue, the suction force would be markedly less than the group that had a foam manifold (Group 5) and therefore the two groups are not comparable in terms of the amount of suction applied to the wound. It is therefore not possible to conclude, based on these results, that suction has no direct influence on vessel formation.

In order to truly evaluate whether hypobaric pressure alone can increase the vascularity (number of vessels) of tissues (without the use of an interface dressing), it would be a requirement that the occlusive dressing be non-collapsible/rigid; this would, in effect, be similar to a bell jar. Only with such a system in place, can one assume that the tissues are exposed to the same negative atmospheric pressure measured on the gauge and draw appropriate conclusions regarding the effects of suction on vascularity. Khouri *et al.* evaluated one such device, which uses the bell jar principle to generate external expansion of breast tissue using negative pressure.[105] It was demonstrated that the breast parenchyma is permanently enlarged following the application of suction over a mean period of 20 weeks.[105] These volume measurements were done using multiple modalities, including magnetic resonance imaging.

The latter demonstrated that the volume increase was due to a true increase in breast parenchyma and not merely swelling.[105] One can infer from this that there must have been an associated increase in vessel formation to accompany the increased tissue parenchyma. Others have confirmed the increase in breast parenchyma using this modality.[118-121] This adds further evidence against Scherer's conclusion that applying suction forces alone to tissue does not increase vessel formation. However, even though Khouri showed increased tissue formation (with the accompanying vasculature), it does not imply that the hypobaric pressure *per se* was the cause for the increased vessel formation. The increased vascularity

might occur directly as a result of the macrodeformation/tissue expansion caused by the bell jar effect or indirectly in response to the growing volume of tissue. Whether it is the negative pressure acting alone, or its synergy with the interface dressing, that stimulates vessel formation, is not known. It has, however, been demonstrated that the combination of negative pressure and an interface dressing results in an increase in vascular growth factors[50, 98] and a histologically proven increase in vasculature.[98, 122] The fact that tissue in the immediate proximity of the dressing has reduced perfusion,[42, 55, 56, 59, 60] may be a key factor for the observed increased vascularity, as hypoxia is a well-known stimulus for angiogenesis.[123]

1.3.9. Tissue pressure-measuring devices

Although the most reliable methods of measuring pressures within the body has traditionally been some form of manometry,[124] one of the common methods of assessing *interstitial hydrostatic pressure* is with the use of micropipettes, diameters of which are in the order of 0,1µm.[125] These are useful at depths of less than 1mm.[125] However, they are prone to breaking at greater depths or during movement of the subject. Other methods have been developed, such as the wick catheter technique,[80, 124, 126] the wick-in-needle technique[127, 128] and a technique requiring the implantation of a 1.5-mm capsule.[78] Each of these, however, has disadvantages.[125] The wick catheter is vulnerable to the clotting of extravasated blood. The wick-in-needle technique requires custom-made needles with optimum-size ports, and the capsule technique requires the implantation of a capsule for four to six weeks and involves tissue distortion, trauma and an inflammatory response.

As mentioned previously, although some papers have measured pressure beneath a NPWT dressing, they were measuring the air pressure at the wound surface, rather than the actual tissue pressure.[43, 46] Of the three papers that investigated true tissue pressure beneath NPWT,[85-87] all used strain-gauge pressure transducers. These sensors have been demonstrated to provide near-

identical readings to wick-in-needle techniques[125] and are commonly used by neurosurgeons to measure intracranial (fluid or tissue) pressure with accuracy.[129] Disadvantages of the conventional fluid-filled catheters, such as the influence of transducer's position relative to the site being measured and other measurement artefacts, are reduced by using strain-gauge sensors.[129] An additional benefit of strain-gauge sensors, is that their miniscule size (0.7 to 1.2 mm)[66, 130] reduces the risk of infection following insertion.[129] However, even these do not allow for the measurement of discrete pressures within different areas within the tissue. Pressures are likely to vary between arterioles, capillaries, lymphatics, interstitial fluid, etc. However, pressure sensors small enough to measure these individual pressures do not, to the author's knowledge, exist.

1.4. NPWT and perfusion

1.4.1. The physiological response of perfusion to hypobaric pressure

Whether NPWT increases or reduces tissue pressure when suction is applied remains equivocal (see section 1.3.2.). If it were to reduce tissue pressure, the resultant effects on perfusion need to be considered, as these too are debatable. In cases where tissues were exposed to hypobaric pressure using a rigid container, the pressures generated within the tissues were, indeed, hypobaric.[131] It has been shown that such application of hypobaric pressure to a limb results in vascular distenstion,[132] implying an increase in blood flow. This fact has been capitalised upon by Grahn and Heller. Their patented device has been shown to increase blood flow to the hand using hypobaric pressure (± -40 mmHg), following which blood can be either cooled or heated to affect core temperature.[133-136]

Others, however, have not been able to reproduce the findings of Grahn *et al.*, and concluded that the heated blood seemed to stay in the arm to which hypobaric pressure was applied.[137, 138] Indeed, some even questioned the validity

of the results of Grahn *et al.*[139] Studies have demonstrated that when hypobaric pressure (± -40 mmHg) is applied to a limb, a neurogenic reflex, termed the *venoarteriolar response*,[139] results in vasoconstriction,[140-143] conflicting somewhat with aforementioned work stating that hypobaric pressure resulted in vascular distension and increased flow.[133-136]

1.4.2. The physiological response of perfusion to NPWT

Numerous studies have investigated changes in perfusion due to NPWT. There is a considerable amount of conflicting evidence in this regard. The seminal paper by Morykwas *et al.* was the first to investigate perfusion changes due to NPWT[15] and therefore will be discussed in some detail. A needle laser Doppler was used to assess perfusion in subcutaneous tissue and deeper muscle on the backs of pigs.[15] Measurements were taken at 15-minute intervals at suction pressure increments of -25 mmHg (range 0 to -400 mmHg). They found perfusion increased maximally at a suction pressure of -125 mmHg (four times baseline levels),[15] resulting in this being adopted as the universally recommended suction pressure for open wounds at the time. The observed increase eventually declined and reached baseline levels after a period of 5-7 minutes.[15] When the "off" periods during intermittent suction were shorter than two minutes, the peaks of the increased waves of perfusion (when suction was switched on again) were shorter.[15] For this reason, they advocated a five-minutes-on/two-minutes-off cycle when using intermittent NPWT. At a suction pressure of -400 mmHg, however, perfusion was decreased to levels below baseline.[15] It returned to baseline when the suction was switched off.

Although the authors commented that the perfusion readings in the different pressure ranges fitted a bell-shaped curve distribution,[15] these values were unfortunately not published anywhere in the paper to illustrate this fact. They also commented that "similar" findings occurred in both subcutaneous tissues and deeper muscle.[15] Again, what was meant by "similar" is not known as these

values were not tabulated or shown anywhere in the paper. This study raises further questions which are not discussed in the paper. For example, if the reduced pressure generated by NPWT results in a gradient causing blood to be drawn toward the wound,[58, 61, 88, 90, 91, 144, 145] why do greater suction pressures do the opposite? One would have thought that greater suction pressures would draw even more blood to the wound. In terms of physiology, what is unique about -125 mmHg, making it the "peak" of the bell-shaped curve? What causes pressures higher than -125 mmHg to result in a decline in the increased perfusion levels? If the application of NPWT at -125 mmHg results in a pressure gradient that increases blood flow, what is the physiological explanation for the observed gradual decline over a five- to seven minute period, eventually reaching baseline (during suction)?[15]

In another component of the above-mentioned study, the survival of random-pattern skin flaps undergoing NPWT was investigated.[15] The authors raised random-pattern skin flaps on the backs of pigs, measuring 3x12 cm. This long length-to-breadth ratio was designed so that the distal portion of all the flaps would undergo some degree of necrosis. The authors would then be able to investigate whether NPWT could reduce the amount of necrosis. The authors divided the flaps into four groups: (1) the flap was exposed to both pre- and post-operative NPWT; (2) the flap was exposed to pre-operative NPWT only; (3) the flap was exposed to post-operative NPWT only; (4) the flap was not exposed to NPWT (control group). Based on the amount of flap discolouration and necrosis, surface area was calculated to determine which flaps contained the most viable tissue. They concluded that the pre-and post-treated flaps had the greatest survival, followed by the post-treated flaps and then the pre-treated flaps; the control flaps fared the worst.[15] Although means and standard deviations were given, the data of each individual flap was not shown anywhere in the paper. Furthermore, in the figure depicting (some of) the flaps, the ones that the authors mentioned fared better appeared to have larger areas of necrosis than the ones that apparently

did the worst (Fig. 2). These photographs raise questions about the subjective nature of this assessment.

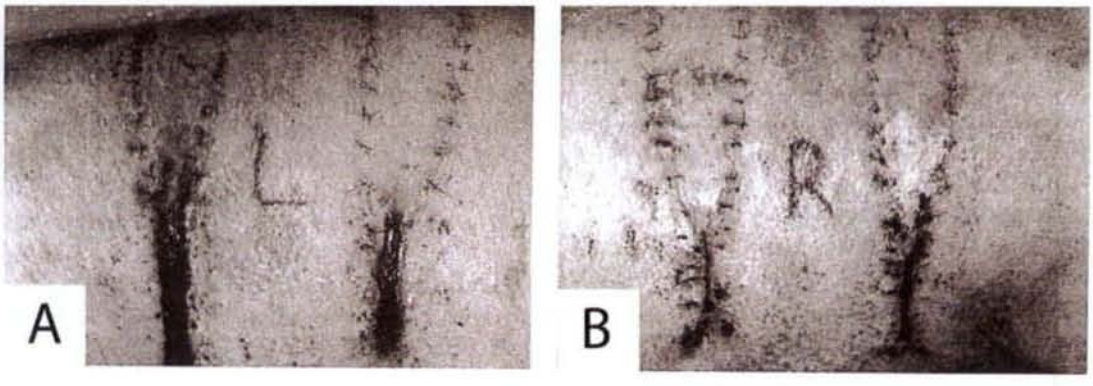

Fig. 2: Photographs depicting some of the flaps in Morykwas' study. (A) The flap on the left had NPWT before and after it was created, whilst the one on the right only had NPWT before creation. Notice the seemingly larger area of necrosis on the left flap, despite this flap being portrayed as faring better than the right. (B) The flap on the left had no NPWT (control), while the flap on the right was treated with NPWT post-creation. Despite the left flap appearing healthier in this photograph, it was documented as faring worse than the right one. (Reprinted with permission from Morykwas MJ, Argenta LC, Shelton-Brown EI, *et al.* Vacuum-assisted closure: A new method for wound control and treatment: Animal studies and basic foundation. *Ann Plast Surg.* 1997;38:553-562.)

Numerous other studies examining the effects of NPWT on perfusion conflict not only with those of Morykwas *et al.* but with one another too. In a study by Timmers *et al.* NPWT was applied to intact forearm skin of healthy volunteers and perfusion was measured with non-invasive laser Doppler probes, which were incorporated into the foam.[146] Whereas Morykwas *et al.* found that the observed increase in perfusion gradually decreased at suction pressures greater than -125 mmHg (eventually being less than baseline levels at -400 mmHg),[15] Timmers *et al.* found that perfusion was increased at all suction pressures tested, even those as high as -500 mmHg.[146]

Researchers from the University of Lund in Sweden have published the largest volume of work relating to perfusion changes due to NPWT. Many of their studies conflict with the findings of both of the aforementioned studies. All their studies, except one,[61] made use of laser Doppler to assess perfusion. In two of these studies, both by Wackenfors *et al.*,[59, 60] it was found that there was an increase in perfusion (supporting the findings of Morykwas *et al.* 15 and Timmers *et al.*[146]) a few centimetres from the wound edge. However, unlike the aforementioned studies,[15, 146] Wackenfors found that there was a zone of hypoperfusion in the immediate proximity of the wound at all levels of suction pressure (-50 to -200 mmHg).[59, 60] The zone of hypoperfusion observed by Wackenfors *et al.* was larger when the amount of suction increased and was also larger in subcutaneous tissue than it was for muscle at the same suction pressure.[59, 60]

The work of Wackenfors[59, 60] therefore contradicts the findings of Timmers *et al.*,[146] who found an increase in perfusion at the foam-skin interface at all suction pressures tested (-25 mmHg to -500 mmHg). It is not known whether or not this disparity can be attributed to the fact that these studies are not directly comparable since Timmers' work was carried out on the intact skin of humans,[146] whereas Wackenfors' experiments were undertaken on the open wounds of pigs.[59, 60]

The work of Wackenfors *et al.*[59, 60] also conflicts with that of Morykwas *et al.*,[15] who also inserted laser Doppler probes "adjacent to the wound" in pigs but found an increased perfusion at the same pressure range in which Wackenfors *et al.* found a decrease in perfusion. However, whereas Wackenfors' reference to "adjacent to the wound" is quoted as being 0.5 cm,[59, 60] Morykwas does not indicate what distance the probes were from the wound.[15] From the work of Wackenfors, it is clear that distance from the wound edge is an important factor when assessing perfusion in the presence of NPWT.[59, 60] As Morykwas does not provide a definition for "adjacent to the wound", it is difficult to draw parallels between their findings and this may explain their conflicting results.

In another two studies from the Lund group, both by Borgquist *et al.*,[55, 56] perfusion studies were conducted on pig wounds, again using laser Doppler needles at varying distances from the wound. They found that perfusion at 2.5 cm from the wound edge progressively increased with increasing suction pressure. However, from -80 mmHg to -125 mmHg the increase in perfusion was not statistically significant. The implication of this study, although not specifically stated by the authors, was that the universally recommended negative pressure of -125 mmHg was not necessarily the optimal pressure to use, because -80 mmHg gave a similar increase in perfusion without the potential of pain due to excessive pressure. However, it must be borne in mind that the recommended pressure (-125 mmHg) was derived from the studies of Morykwas *et al.*, where foam was used, whereas Borgquist used gauze-based dressings. It may be that, for a given suction pressure, gauze and foam do not create the same tissue pressures or perfusion changes and it is therefore not necessarily correct to deduce from the studies of Borgquist *et al.*[55, 56] that -80 mmHg is as optimal/beneficial as -125 mmHg.

One must also consider that measuring changes in perfusion adjacent to the wound does not necessarily give an indication of the changes in perfusion beneath the foam at the wound base, as the compressive forces of the foam may be entirely different here. Yet, it is in this area where knowledge of perfusion changes is most important, as this is where wound healing takes place. This was, in fact, demonstrated by Greene *et al.*, who showed significant histological differences in areas of the wound which were not in contact with the interface dressing.[98]

In one of the few studies on the effects of NPWT on perfusion conducted without the use of laser Doppler, Chen *et al.* demonstrated increased perfusion due to NPWT.[91] These investigators used a microcirculation microscope and video-imaging software to assess perfusion.[91] Skin perfusion was evaluated at a distance

of "about 0.5 cm" from a wound undergoing NPWT, on the dorsal surfaces of rabbit ears. They accomplished this by removing a 1-cm diameter circle from the adhesive drape "about 0.5 cm from the wound". It was found, among others, that NPWT at -10, -15 and -20 kPa (-75, -113 and -150 mmHg respectively) increased perfusion, flow velocity and also capillary calibre.[91]

In a similar study, Ichioka *et al.* used video-imaging software to directly visualise and determine perfusion in wounds created over the gluteal areas of rats.[147] They found that perfusion was increased at -125 mmHg but reduced at -500 mmHg (only these two suction pressures were tested).

Again, the methodology of both of these studies raises questions. Both of these studies claimed to be assessing perfusion of the wound bed undergoing NPWT.[91, 147] However, the wounds they spoke of were not underneath the NPWT dressing but alongside it, and it is unlikely that this area experiences the same physiological changes that occur directly beneath the NPWT interface dressing. Further confounding factors in the studies of Chen *et al.*[91] and Ichioka *et al.*,[147] are that the effect that a given suction pressure has on tissue pressure and perfusion is unlikely to be equivalent in different anatomical locations[59, 60] and in different animals. It is, therefore, not known if the results of Chen *et al.* [91] and Ichioka *et al.*[148] can be compared to those of the aforementioned porcine studies.[15, 55, 56, 59, 60]

Kamolz *et al.* investigated the effects of NPWT on perfusion in burned hands, using indocyanine green video angiography.[149] Perfusion was significantly increased in the hands exposed to NPWT after two to three days. Along with the previously discussed two studies,[91, 148] this is one of very few to investigate perfusion changes (as a result of NPWT) without the use of laser Doppler. Unfortunately this study does not provide information on how NPWT affects perfusion when suction is applied, as the images were taken with the dressings off.

Lindstedt *et al.* demonstrated that coronary artery blood flow is increased by NPWT using electromagnetic flow meter probes.[61] However, the probes were placed around the roots of three coronary arteries, which were not directly beneath the NWPT dressing. These areas of measurement were therefore not exposed to the compressive forces of the foam; on the contrary, they were exposed to the hypobaric pressures that were created within the chest cavity.[90] This may be an explanation for the observed increased perfusion in these vessels.

1.4.3. Optimal suction pressures and effects on perfusion

Considerable debate has been generated regarding the correct suction pressure to use when utilising NPWT.[21, 42, 56, 58-60, 88] In earlier descriptions of a form of NPWT (the "Kremlin papers") two of the four papers recommended pressures ranging from -75 to -100 mmHg,[17, 19] with tissue oedema being reported at pressures of -120 mmHg[19] and fresh haemorrhages at -160 mmHg.[19] The other two papers recommended considerably higher pressures, in the order of -0.8 to -1.5 ATA and did not report any untoward events (interestingly a suction pressure of -1.5 ATA does not exist, as it is not possible to produce more than -1 ATA).[18, 20] All four were clinical observational studies and did not assess perfusion.

Furthermore, it appears that these studies used a rigid container to apply the NPWT. Although this *will* induce hypobaric tissue pressures, the current embodiments of NPWT are essentially collapsible containers and, as a result, do not necessarily generate hypobaric tissue pressures. On the contrary, two[85,86] of the three papers[85-87] that studied tissue pressure beneath NPWT, reported hyperbaric tissue pressure as a result of NPWT. The fact that the Kremlin papers' incarnations of NPWT (rigid containers) were likely to create negative tissue pressure is probably what accounted for their findings of tissue oedema or even haemorrhages when the suction pressure was increased.[35] NPWT dressings used in the present day (which are collapsible) have been shown to reduce oedema[91, 150] and tamponade superficial bleeding.[151] Further discussions on optimal suction

pressure will therefore only refer to studies conducted with current forms of NPWT (collapsible).

Chariker *et al.* recommended a suction pressure of -60 to -80 mmHg, although did not give reasons for their selection.[35] This was also a clinical outcomes study and did not investigate perfusion. Following the key papers of Morykwas *et al.*[15] and Argenta *et al.*,[14] and the commercialisation of the V.A.C. device, the world-wide consensus was that -125 mmHg was the optimal suction pressure for most wounds.[94, 152] The two seminal studies[14, 15] included clinical findings and also experiments on perfusion, supporting the use of -125 mmHg. The K.C.I. guidelines[94] recommended lower pressures (-75 to -125 mmHg) on skin grafts and higher pressures (-125 to -150 mmHg) for flaps, although little evidence for these recommendations exists. The bulk of all published work investigating NPWT has utilised a suction pressure of -125 mmHg.

Until 2009, all studies researching the effects of NPWT on perfusion used PU foam.[42] More recently, however, an increasing number of studies using the Chariker-Jeter technique have appeared.[41-46, 48, 53-56] With this technique the recommended suction pressure is -80 mmHg, based on the paper by Chariker *et al.*, despite the fact that no evidence was given by these authors for the selection of this pressure.[35] Subsequent studies have bolstered support for the use of these suction pressures, claiming that there is no significant increase in perfusion beyond -80 mmHg.[42, 54, 56, 59, 60] However, the fact that gauze is used as an interface dressing in these studies should be taken into account, as this may be the cause for observing the different effects on perfusion, as opposed to when foam is used.

It has also been demonstrated that the changes in perfusion as a result of gauze-based NPWT are not significantly different to those using foam-based NPWT. This was demonstrated by Malmsjo *et al.*, in the only comparative study published that evaluates the effects on perfusion of gauze- and foam-based NPWT.[42] In this

study perfusion was assessed with laser Doppler at varying distances from the *wound edge* and also vertically from the *wound bed* (depth), at different suction pressures. Interestingly, these authors made their conclusion based on perfusion changes in the subcutaneous tissues 0.5 cm away from the dressing (tissue surrounding the dressing) at a suction pressure of -75 mmHg.[42] The specific values obtained at a depth of 0.5 cm into the *wound bed* (where wound healing takes place) were not commented on in the paper. However, their graphs of these values demonstrate a noticeably greater reduction in perfusion beneath foam (> 50%) compared to gauze (± 30%).[42] This would imply that there is, in fact, a difference in the effect that gauze- and foam-based NPWT has on perfusion.

Regarding depths of 1 cm into the wound bed, the authors commented, "There was a suggestion that the reduction in wound bed blood flow penetrated into deeper tissues under PU foam than gauze..."[42] Further scrutiny of these graphs reveals that, at a depth of 1 cm into the wound bed there appears to be a clear *reduction* in perfusion in foam-based NPWT, while there appears to be an *increase* in perfusion in the gauze-based NPWT. Again, these observations conflict with the authors' conclusions that gauze- and foam-based NPWT results in similar changes in perfusion.

1.4.4. Perfusion-measuring devices

1.4.4.1. Laser Doppler

Three studies have evaluated perfusion using modalities other than laser Doppler.[91, 147, 149] These measuring modalities (microcirculation microscope and video-imaging software,[91, 147] and indocyanine green videography[149]) all require a direct line of sight to the tissues. Therefore they cannot be used to evaluate the wound bed undergoing NPWT with the dressing still in place. The tissues alongside the dressing can be evaluated while the dressing is in place but this

is not a true reflection of perfusion changes beneath the dressing and therefore these techniques are seldom used.

The overwhelming majority of research conducted on perfusion changes due to NPWT has used laser Doppler[15, 42, 55, 56, 59-63, 88, 146, 153-156] and some consider this to be the gold standard in other varieties of perfusion research too.[157] The laser Doppler measurement of perfusion (perfusion units) is an arbitrary unit that is derived by multiplying the velocity and concentration of red blood cells within a tissue volume (less than 1 mm^3).[59, 60, 158, 159] These two parameters are determined using monochromatic light.

This laser light is carried to the tissues via one optical fibre and the reflected light is received via another optical fibre, which transports it back to monitor.[160] The light entering the tissues scatters as it encounters various types of cells and substances (proteins, keratin, melanin, haemoglobin etc.) with varying optical properties.[160] When this light encounters a moving red blood cell it undergoes a wavelength shift explained by the Doppler effect, while the static objects in its path do little to change its wavelength.[59] The wavelength changes are therefore affected primarily by the concentration and velocity of red blood cells.[59] It has been suggested that the velocity parameter provides a more direct measure of physiological changes than the concentration parameter.[159] Numerous probes can be used and include non-invasive and invasive varieties. The most suitable for measurement of perfusion changes beneath NPWT dressings is the invasive variety, as these fine filaments can be passed with little trauma *into* the tissue beneath the dressing. On the other hand, placing external probes underneath the interface dressing, *on top* of the wound, would result in the probe being pushed into the tissues when suction is applied, which could interfere with the readings.

The laser Doppler has shortcomings, however: firstly, its reproducibility is not always consistent. In addition it is subject to motion artefacts, it lacks quantitative

units, and there is no information on the volume of tissue sampled.[160] Furthermore, the flux signal recorded by a laser Doppler never falls to zero, even when evaluating vessels with no flow.[160, 161] This is likely to be the result of the Brownian motion of cells in static blood, vasomotor activity and electrical noise.[160, 161]

Due to the potential for motion artefacts, laser Doppler research ought to be undertaken with the probe completely immobile, with the only changing variable being the velocity and quantity of blood flowing through the vessels. Due to the dynamic nature of NPWT dressings, this immobility cannot be reliably achieved when suction is applied. It could be argued that the laser Doppler is, therefore, not suitable for measurement of perfusion changes in NPWT research. In addition to this, other variables are introduced during NPWT, which are not accounted for. For example, the macrodeformation that the NPWT dressing generates is likely to change the specific area of tissue being evaluated (before and after suction is applied). Capillary beds could become denser, as more tissue is compacted into a smaller area, thereby increasing the concentration of red cells measured (or the opposite could occur depending on whether tissues are distracted or compressed).

Furthermore, the macrodeformation may shift the focus of the probe, so that an entirely different cubic millimetre of tissue is being measured to the original one prior to application of suction. The macrodeformation may also compress vessels, resulting in a reduced diameter. Blood attempting to pass through a narrower vessel will have an increased velocity, although not necessarily an increased flow.[162] As velocity is one of the parameters used to determine perfusion, this may result in the laser Doppler producing a falsely elevated value for perfusion, despite the actual perfusion potentially being reduced. These proposed measurement flaws of the laser Doppler, particularly relevant to NPWT research, have not formally been investigated.

1.4.4.2. Transcutaneous partial pressure of oxygen ($tcpO_2$) sensors

Measurement of tissue oximetry or transcutaneous partial pressure of oxygen is another technique utilised in various fields as an indirect, yet reliable, measure of tissue perfusion.[163-165] It is one of the few non-invasive techniques to monitor tissue oxygen tension, which is a reflection of perfusion.[164] The technique was pioneered 40 years ago[165] and is a modification of the invasive polarographic electrodes used in the 1940's.[163] A fixation ring filled with contact solution is attached to the skin. An electrode with a heating element and thermistor (to maintain a preset temperature of 42°-45°C) is attached to the fixation ring. Heating the skin surface dilates capillaries, opens skin pores, decreases oxygen solubility and shifts the oxyhaemoglobin curve to the right.[163] This facilitates oxygen migration to the skin where it can be analysed. It is most commonly used to predict non-responders for hyperbaric oxygen therapy and also to determine amputation sites in peripheral vascular disease.[163] Its disadvantage is that it cannot be used on wounds, as the fixation ring cannot attach to moist tissues and is therefore always used alongside the wound.

$TcpO_2$ has never been used in the field of NPWT research. This may by attributed to the fact that the sensor cannot be placed beneath the dressing to assess perfusion, as the collapsing dressing would force the sensor onto the tissues and thereby influence the underlying perfusion. In addition, the hypobaric pressure within the dressing would withdraw the contact fluid lying in the fixation ring and thereby influence the results. In theory, however, it can be placed alongside the dressing to assess adjacent perfusion or tissue oxygen tension.

1.4.4.3. Thermographic imaging

Thermography is based on the principle of the Stefan-Boltzmann Law, whereby the energy flux emitted by a surface is related to its temperature. All matter emits radiant energy or thermal radiation as a consequence of its absolute temperature. In human skin, the amount of radiation emitted is proportional to

the change in temperature of the skin surface, which is proportional to change in microcirculation blood flow.[166] Importantly, skin heating is dependent on both external and internal factors, such as heat delivery by blood flow and conduction properties of the subcutaneous structures. External factors such as radiation, convection, and conduction must be closely controlled in order to ensure good accuracy of thermography. Thus under thermally neutral conditions skin-surface temperature is controlled only by the blood flow rate of the cutaneous tissue.[167] Consequently, changes in blood flow may be estimated by measuring temperature variation via thermography.

Thermography has been found to be a good modality for accurately measuring skin blood flow[168] and has found utility in many other scenarios, both clinical and experimental. These include the measurement of blood flow in burns,[169] atherosclerosis,[170] peripheral vascular disease[171], varicose veins[172] and many other disease states.[173-175] The main advantages of thermography are that it is relatively easy to use, is a non-contact device, has good spatial resolution and can map temperature distribution across regional surfaces of the body.[176] One disadvantage particularly relevant to NPWT research is that it cannot assess perfusion change beneath the interface dressing.

1.4.4.4. Radioisotope perfusion imaging

Another potential modality to measure perfusion is radioisotope perfusion imaging. Despite its disadvantage of requiring the intravenous injection of radioactive isotopes, it is still used for a variety of therapeutic and diagnostic procedures.[177] The diagnostic utility of this modality is mostly found in peripheral vascular disease[177] or cardiac pathology.[178] Its use in determining skin perfusion can be confounded by the detection of underlying muscle perfusion by the gamma camera.

In NPWT, it could however, find a use in assessing perfusion changes as a result of circumferential NPWT. In particular, this modality will be useful for determining whether perfusion increases or decreases when suction is applied to the dressing. Whether the camera is detecting skin or muscle perfusion changes is less relevant in such a scenario.

The fact that no sensor is needed to be placed into the dressing is the greatest benefit of this modality. Another advantage is that it can assess what changes occur *beneath* the dressing, as opposed to many other modalities that require their sensors to be placed alongside the dressing. Like the thermal imaging camera, this modality also allows for the assessment of perfusion changes in an entire limb, rather than the point measurements made by other modalities, for example, laser Doppler.

1.5. Gaps in the literature and the need for further research

Throughout its history, NPWT has been confounded with conflicting statements regarding, amongst others, suction pressures,[21, 179, 180] resultant effects on tissue pressure[85-87] and changes in perfusion.[15, 42, 56, 58-60, 88, 91, 146, 147, 149, 179] Not surprisingly, the mechanism of action of NPWT remains, to a large extent, a mystery.[6, 7, 21, 59, 60]

The primary starting point in attempting to make sense of these controversies should be to clarify and understand the physics of these dressings. This process must begin with the confirmation of whether the immediate action of NPWT dressings is to increase or decrease the underlying tissue pressure. This would lay the foundation for further research. Without this basic knowledge we cannot progress to the more complicated studies, which have been undertaken previously, such as those on blood flow. Determining this basic fact can be done *in vitro* initially but should ultimately be done *in vivo* – ideally in tissues that have compliance and elasticity similar to our own.

Once this is known, the effects that these tissue pressure changes have on perfusion should be evaluated *and* these findings should correlate with the tissue pressure findings. If the tissue pressure is increased, then one would expect perfusion to be decreased. This is, after all, the basic underlying principle of applying a tourniquet or a pressure dressing; these serve to increase tissue pressure and reduce blood flow.

The large number of conflicting studies on perfusion using laser Doppler and the previously mentioned potential flaws of this modality (when used in NPWT research), encourages further research to be undertaken using another modality. Ideally different modalities should be utilised; however, regardless of the modality chosen, it should be a pre-requisite that it can measure perfusion while the dressing is still in place.

Lastly, the laser Doppler's potential flaws should be further investigated. The previously mentioned theory, that a measuring error may occur as a result of changes of blood velocity in vessels that are compressed, is based on sound scientific principles. This theory, however, must be put to the test. This will help to determine the relevance of prior research using this modality and its future utility in any further research on NPWT.

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2 *Evolution and Aims of the Research Components of this Thesis*

Outline

2.1. Introduction

The studies presented in this thesis represent the evolution of a concept, which originated as a simple question in 2006. The question was raised following the unfortunate outcome of a patient who had a partial hand replant. The patient in question was thrown from a moving train and sustained a near-total amputation of half of his hand and wrist (Fig. 3). Both radial and ulnar arteries were severed.

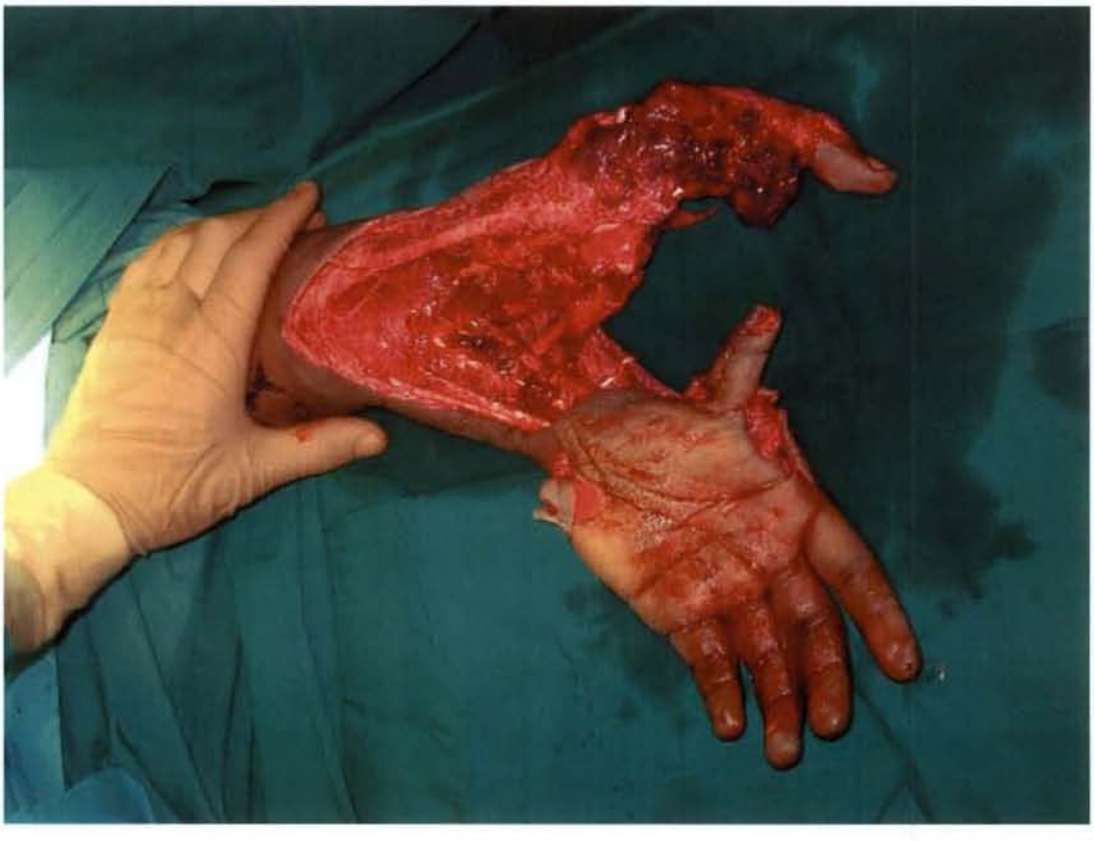

Fig. 3: A near-total amputation of a hemi-hand with both radial and ulnar arteries severed.

Following successful replantation of the hemi-hand, the tissues appeared to be perfusing satisfactorily. After five days, a decision was taken by a registrar to apply a circumferential negative-pressure wound therapy (NPWT) dressing to the hand to increase granulation tissue in an area on the dorsum where skin was missing. After 48 hours the dressing was removed and, much to the horror of the registrar, the hand was necrotic.

It could be argued that this was an extensive injury with a high risk of anastomotic failure but for this to have occurred after five days is unusual. This raised the question of whether the NPWT dressing was the culprit. There were questions raised regarding whether NPWT could compress tissues and reduce perfusion but it was pointed out that it is generally known that NPWT reduces tissue pressure and increases perfusion.

Having done a cursory literature search, the principal investigator of this thesis found nothing that would support either of the two opinions on tissue pressure. An attempt was therefore made to resolve this disagreement by conducting an experiment ("Vacolitre experiment", see Chapter 3, section 3.3.1.), which would hopefully answer the question. What started as a simple investigation, led to a series of experiments, which ultimately inspired the original research studies presented herein. This domino effect was because the findings of the preliminary studies raised new questions, which instigated the conception of more studies, each raising even further questions. At the conclusion of this research, even though still newer questions remain to be investigated, a paradigm shift in our understanding of the biomechanics of NPWT and its effects on tissue pressure and perfusion was demonstrated.

2.2. Preliminary research

A series of informal studies were undertaken to investigate various questions raised regarding the biomechanics of NPWT and its effects on tissue pressure. These findings implied that NPWT is likely to increase tissue pressure, which conflicts with the common perception that NPWT creates a pressure gradient which will create a surge of blood flow to the wound.[1-7]

2.3. In vitro *pressure experiments*

As a result of the preliminary research it was realised that a formal, well-designed study on how NPWT affects the underlying tissue pressure was mandatory. These experiments served merely to confirm or refute the findings of the preliminary research (that NPWT increases tissue pressure) and therefore were conducted on inanimate substances. This research confirmed the preliminary studies' findings.

2.4. Human in vivo *pressure experiments*

The *in vitro* pressure experiments demonstrated findings which conflicted with common opinion. Questions were raised regarding the validity of extrapolating these findings to living tissues. Living tissues comprise various components, the substances of which may be in fluid, solid or malleable form. These may respond differently to the application of NPWT.

Therefore a formal study, which had a similar structure to the *in vitro* study, was undertaken on living human tissues. The outcomes of this study confirmed those of the *in vitro* study.

2.5. Circumferential NPWT and perfusion

The fact that NPWT increases tissue pressure is incongruous with the fact that it increases perfusion. Either the studies on tissue pressure were incorrect or the published research on perfusion was flawed. The evidence to support the fact that NPWT increases perfusion, however, is substantial.[1, 2, 6, 8-17] On the other hand, the findings of the two formal pressure studies were unequivocal. Furthermore, they also concurred with the findings of the German authors, Maier *et al.*[18] and Willy *et al.*[19]

Due to the potential that the perfusion studies may have been flawed (discussed in detail in Chapter 1), it was realised that perfusion may need to be reassessed using alternative modalities. A study was therefore undertaken to determine perfusion beneath circumferential NPWT using radioisotope imaging. This demonstrated that perfusion was, in fact, reduced, concurring with the findings of the prior two studies.

2.6. Non-circumferential NPWT and perfusion

Despite that fact that the *in vitro* and *in vivo* studies demonstrated that tissue pressure is increased in both circumferential and non-circumferential NPWT, there remained an element of doubt regarding the perfusion study on circumferential NPWT. Even though the dressings were applied in a manner that avoided constriction (sandwich technique), it was still felt that tissue constriction may have played a role in the observed decrease in perfusion.

Therefore a study was undertaken to evaluate perfusion in non-circumferential NPWT. In this study too, however, it was demonstrated that NPWT reduces perfusion, even when applied in a non-circumferential manner.

2.7. Thermography study

Following the publication of the aforementioned studies, researchers who had conducted perfusion research using laser Doppler pointed out that they too found that perfusion was decreased in close proximity to the dressing but that it was increased a couple of centimetres away.[12-16] They concluded that this was in keeping with the tissue pressure studies and that the increased pressure within tissues in close proximity to the dressing may be the cause for the reduced perfusion. However, they maintained that the perfusion is increased a couple of centimetres away.

Although the tissue pressure studies showed that the increased tissue pressure dissipated rapidly as the distance from the NPWT dressing increased, these pressures were still hyperbaric up to 3 cm from the dressing (in the *in vitro* studies), never becoming hypobaric. Therefore, the findings that there was increased perfusion a couple of centimetres away were questioned, particularly in light of the recent doubts cast on the laser Doppler's accuracy in this setting.

A thermography study was undertaken to determine if such a zone of increased perfusion does, indeed, exist. This study could not find evidence of a zone of increased perfusion.

2.8. Laser Doppler study

Sufficient evidence to support the fact that NPWT increases tissue pressure and reduces perfusion was therefore developed. The findings of the laser Doppler, however, are in contradistinction to this. Considering that the overwhelming majority of studies on perfusion changes due to NPWT have been conducted using laser Doppler, it became apparent that the cause for this discrepancy needed to be investigated.

A theory was proposed, which could explain the paradoxical findings of the laser Doppler. The theory was tested in this study and shown to be correct. The implications of this are far reaching. It not only invalidates prior laser Doppler research on NPWT but it highlights the fact that some of the proposed indications for NPWT may be contraindications - perhaps even life threatening. An example is the proposal that NPWT can be used to augment perfusion in ischaemic myocardium.[10, 11, 20-23] Until this can be confirmed using a variety of modalities, this proposal ought to be abandoned.

2.9. The manufacturers' influence on NPWT research.

Numerous studies comparing the outcomes of the two commonly used forms of NPWT (V.A.C. and Chariker-Jeter systems) were studied to determine if the level of manufacturer involvement in these studies correlated with the outcomes. It was found that the outcomes of these studies almost always proved beneficial to the manufacturer involved in the study. This may be an additional confounding factor in the quest to understand the mechanism of action of NPWT.

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3 Preliminary Research

Outline

3.1. Introduction

A series of experiments were conducted which later led to the formal studies. It must be stressed that all of these were informal studies and were not necessarily conducted according to rigid scientific principles, nor did the outcomes necessarily result in statistically significant findings, as these were not statistically tested. Despite the informal nature of many of these studies, some insight was gained from the observations made and they will therefore be presented as a prequel to the formal studies.

Due to the nature of these studies, they are presented in an informal manner. As many of the results merely added insight, rather than definitive data, the conclusion of this chapter will not affirm definitive facts, but summarise the impressions gained from these experiments instead. The impressions are speculative and do not necessarily imply factual evidence but rather the principal investigators' own opinions to explain many of the observed findings. Many of these experiments were informally discussed with two physicists (Professors Margit Harting and David Britton) at the University of Cape Town. The abstract nature of the physics involved in NPWT and the mathematical explanations for observed findings are not readily covered in physics textbooks, as most mathematical models on hypobaric pressure involve rigid containers (unlike a NPWT dressing). Many discussions with the physicists were left open-ended pending further research and more mathematical analyses.

3.2. Perceptions of the effects of NPWT on tissue pressure

This survey aimed to determine what peoples' impressions were of how NPWT affects tissue pressure. It was targeted at people who regularly use NPWT or those who ought to have a good understanding of physics and/or pressures within the human body.

The following groups of people were individually interviewed in the survey (N=30):

- Two professors of physics
- Two mechanical engineers
- Two biomedical engineers
- One professor of neurosurgery
- Five plastic surgeons
- Five orthopaedic surgeons
- Five general surgeons
- Five anaesthetists
- Three wound-care nurses

The following question was posed to the candidates:
"If you apply a non-circumferential NPWT dressing to a leg and apply suction at -125 mmHg, will the underlying tissue pressure: (A) immediately decrease; (B) stay the same as atmospheric pressure; or (C) increase?"

In reply to this, twenty-three felt that the tissue pressure would decrease, three felt that it would stay the same as atmospheric pressure and five thought it would increase (Fig. 4).

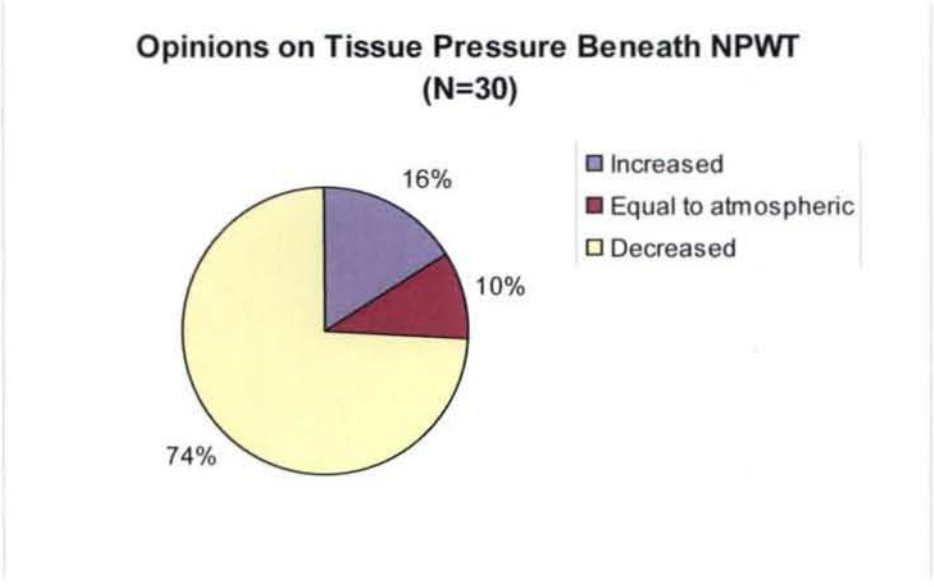

Fig. 4: Pie chart illustrating the proportion of differing opinions on how NPWT affects tissue pressure.

From this it could be seen that the majority of people interviewed expected that NPWT would reduce tissue pressure. About a quarter, however, differed from this opinion, warranting further research to clarify this question.

3.3. Pressure studies

3.3.1. Vacolitre experiments

This was the very first experiment, the results of which inspired the series of experiments that culminated in this Ph.D. research project. The aim of this test was to clarify whether applying circumferential suction to an object using a NPWT dressing would reduce or increase the pressure within the object (if the object's pressure changed at all). In other words, this experiment was designed to test whether a circumferential NPWT dressing applies force to the underlying substance, and if so, whether this force is compressive or distracting in nature.

An "off the shelf" NPWT dressing was utilised using generic foam, adhesive occlusive drape and suction tubing. The substance to undergo NPWT was a one-litre intravenous infusion bag ("Vacolitre"). The content of the bag was connected to plastic tubing, which was filled with water to create a manometer. Therefore, the object that would be subjected to NPWT (the Vacolitre in this case) was also the pressure-measuring device. The fluid in the Vacolitre was tainted with methylene blue to allow for easier visibility of the meniscus of the manometer. The plastic tubing was taped vertically to a wall above the Vacolitre.

Four different circumferential NPWT configurations were tested (Fig. 5) because the findings of each experiment inspired a subsequent test with a different design. The slabs of foam in all instances were exactly the same size (20x25 cm).

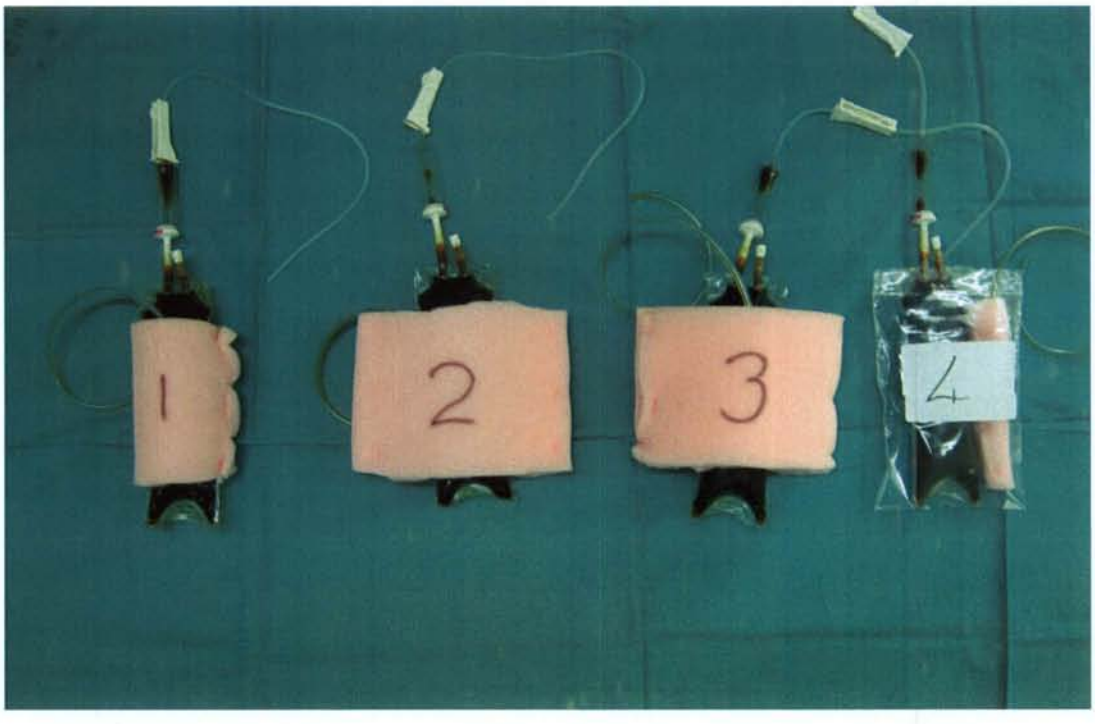

Fig. 5: Four different types of circumferential NPWT dressings applied to Vacolitre bags.
1 - Wrap-around technique, 2- Sandwich technique, 3 - Double-layer sandwich technique, 4 - No foam technique.

A suction pressure of -200 mmHg was applied in each experiment using conventional hospital wall suction. After switching the suction off and waiting five minutes for the foam to fully re-expand, the suction was reapplied. This was repeated five times. The different experiments and their results will be briefly discussed:

(A) Wrap-around technique

In the first test a single slab of foam was loosely wrapped around the Vacolitre and the dressing was completed following the conventional technique using the adhesive occlusive drape, with the suction tubing in contact with the foam.

On application of suction the pressure within the bag increased (mean, 17 cm H_2O) This raised the question of whether the foam was constricting the Vacolitre when it contracts under suction, thereby resulting in an increase in Vacolitre pressure, which is not necessarily a direct result of the suction. If the shortening of the foam slab caused constriction, then loosely sandwiching the foam between two separate slabs of foam ought to prevent this constriction and perhaps result in a lesser, or even hypobaric pressure. This theory inspired the next experiment.

(B) Sandwich technique

In attempt to eliminate the possibility of the foam constricting the Vacolitre, it was decided to utilise a sandwich configuration, whereby two slabs of foam (each the same size as in the previous test) were placed on either side of the Vacolitre. The adhesive occlusive drape was applied in a similar manner, with two sheets of drape placed on either side of the foam and the periphery of the drape being allowed to stick to its contralateral partner to effect a seal. Suction tubing was placed in contact with the foam prior to this.

Contrary to the hypothesis that the sandwich technique will eliminate the increased pressure within the bag, it was found that the opposite occurred. On application of

suction, the pressure in the bag rose even higher than in the previous experiment (mean, 21 cm H_2O). This finding was difficult to explain. As all parameters were equal to the previous experiment, except the amount of foam used (two slabs instead of one), it was hypothesised that the amount of foam used may play a role in the magnitude of pressure change within the bag. This inspired the next experiment.

(C) Double-layer sandwich technique

In an attempt to evaluate the pressure changes when using more foam, double the amount of foam was used in this experiment. Four slabs of foam, each identical in size to the previous experiments, were used. Two slabs were placed on either side of the Vacolitre bag in order to complete another sandwich NPWT dressing, using the above-mentioned technique.

On application of suction the pressure within the bag increased more than in all the other experiments (mean, 34 cm H_2O). Double the amount of foam did not double the pressure increase however. This raised the question of whether having a NPWT dressing without foam would affect pressure within the bag. In other words, would suction alone be able to increase the pressure within the bag? This question gave rise to the last Vacolitre experiment.

(D) No-foam technique

In this experiment the Vacolitre was placed within a non-adherent plastic bag. The suction tube was placed alongside the Vacolitre, with a small sliver of foam wrapped around the tube only, to prevent the plastic bag from blocking the holes of the tube on application of suction. The top of the bag was sealed off.

On application of suction there was an increase in pressure within the bag (mean, 12 cm H_2O), although less than in all the other experiments. Whether the small sliver of foam was enough to account for the pressure increase or whether suction

per se increased the pressure within the bag remains unanswered. Unfortunately it is not possible to create a NPWT dressing without some amount of interface dressing acting as a manifold to prevent the holes of the suction tube being blocked by the surrounding plastic.

This series of experiments demonstrated that NPWT appears to increase tissue pressure and that a larger volume of foam results in greater pressure increases (Table 1). The NPWT dressings in these experiments were all circumferential, however, which raised the question of whether the same would occur in a flat NPWT dressing. This question gave rise to the next experiments, the "Table Experiments".

Table 1: Vacolitre bag pressure changes following application of various configurations of circumferential NPWT. Larger volumes of foam appear to increase the bag pressure to a greater extent.

Technique	Mean Pressure Change
1. Wrap-around	17 cm H_2O
2. Sandwich	21 cm H_2O
3. Double-layer sandwich	34 cm H_2O
4. No-foam	12 cm H_2O

3.3.2. Table experiments

It could be argued that circumferential NPWT will always tend to increase the underlying substance's pressure, even in the sandwich configuration, because the nature of the dressing dictates that the foam would always assume the smallest size it can and thereby compress the object undergoing NPWT. It can be envisioned that any object preventing this size reduction by being interposed between the collapsing slabs of foam (as is the case with circumferential NPWT), will experience a compressive force and thus undergo an increase in pressure. The table experiment was designed to determine whether there would be any

pressure increase beneath NPWT applied in a non-circumferential manner.

As with the previous experiment, an intravenous infusion bag and generic foam (2-cm thick) were used, but this time a 50-ml bag was used instead of the one-litre bag. In addition, a range of suction pressures were tested (-75 mmHg to -450 mmHg). Negative pressure was generated using a portable suction pump with an accurate pressure gauge (Schuco, Carle Place, N.Y., USA). An arterial pressure transducer (Arrow International, Inc., Reading, PA, USA) was used to measure the pressure changes within the bag. Three experiments were carried out, each inspired by the findings of the previous one.

(A) Single slab of foam

The bag was laid flat on the table and a 22.5x25-cm slab of foam was placed on top of the bag. Suction tubing was placed alongside the bag, rather than on the bag, to prevent pressure increases due to the tube being pushed into the bag by the foam. The configuration was sealed off by applying adhesive occlusive drape over it and sticking it to the table (Fig. 6).

Fig. 6: Non-circumferential NPWT applied over a 50-ml infusion bag. An arterial pressure transducer measures the pressure within the bag.

It was found that as suction pressure increased, so too did the pressure within the bag. This finding, and those of the aforementioned experiments, were postulated to occur due to the application of force by atmospheric pressure external to the NPWT dressing, which was applying this force in an attempt to equalise the pressure differential on either side of the adhesive occlusive drape.

(B) Two slabs of foam (double the volume and double the thickness of the foam in (A))

Following on from the previous experiments on circumferential NPWT, the question was raised about the effects of double the amount of foam on the pressure within the bag. For this reason two layers of foam were used (each measuring 22.5x25 cm).

This experiment demonstrated that the pressure in the bag increased to a greater extent (for equivalent suction pressures) than for the single-layer foam.

(C) Single slab of folded foam (same volume as (A) but double the thickness)

Following on from the proposal put forward at completion of experiment (A), and the results of experiment (B), it was hypothesised that the increased thickness (4 cm as opposed to 2 cm) of the foam may be the cause for the greater magnitude of pressure increase within the bag. This hypothesis was based on the observation that the foam had an inherent resistance to collapse. It was assumed at this stage that, on application of suction, atmospheric pressure on the outside of the dressing was applying a force to the dressing, which would cause the foam to collapse. Therefore, if double the thickness of foam were present, then more force would be needed to collapse the foam. As this force is transmitted to the bag, it could be envisaged that this increased force would be the cause of the increased bag pressure observed in the two-layer foam.

To differentiate whether it was indeed the increased thickness, or the increased volume of the foam that caused the increased bag pressure, a third experiment was done, whereby a single slab of foam was folded onto itself, thereby effectively having double the thickness yet the same volume as experiment (A). If it was the thickness of the foam that caused the greater magnitude of pressure increase within the bag, then this too would result in a similar pressure increase to experiment (B).

The results demonstrated that the pressure increments within the bag were, in fact, almost identical to those of experiment (A), implying that it was not the thickness of the foam but the volume thereof, that was accountable for the greater pressure increments observed within the bag (Fig. 7).

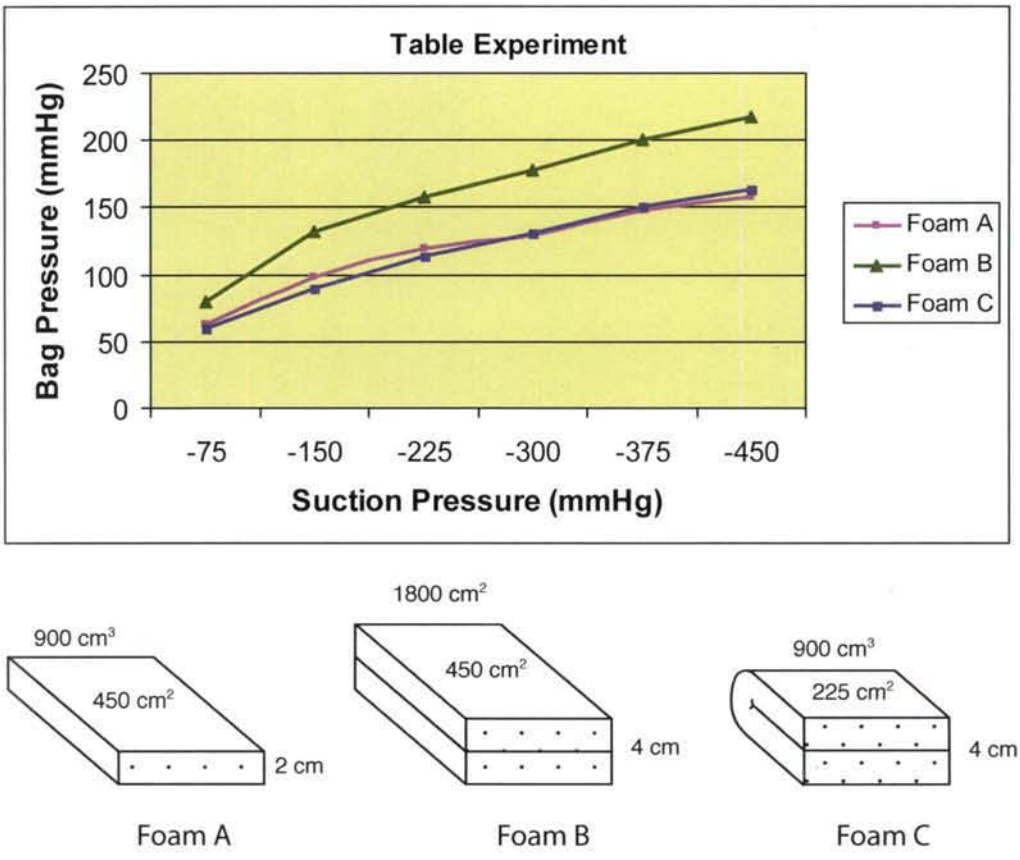

Fig. 7: Graph and diagrams demonstrating three different experimental foam configurations (A, B, and C) and the pressure increments within the underlying bag in response to increasing suction pressure. Identical foam volumes resulted in near-identical pressure increments within the bag, despite double the thickness in foam (C).

The table experiment appeared to demonstrate that non-circumferential NPWT is as capable of increasing underlying tissue pressure as circumferential NPWT. However, the fact that the bag itself prevented the foam from collapsing down onto the table, and effectively was squashed between the foam and table, raised the question of whether this was a true representation of whether non-circumferential NPWT increases or decreases the underlying substance pressure *in vivo*. In other words, in this experiment, the pressures measured were of an object interposed between the foam and its underlying substance (Fig. 8), and not those of the underlying substance itself. This represented a probable flaw in the methodology of the experiment, which could result in incorrect conclusions regarding whether

non-circumferential NPWT increases tissue pressure.

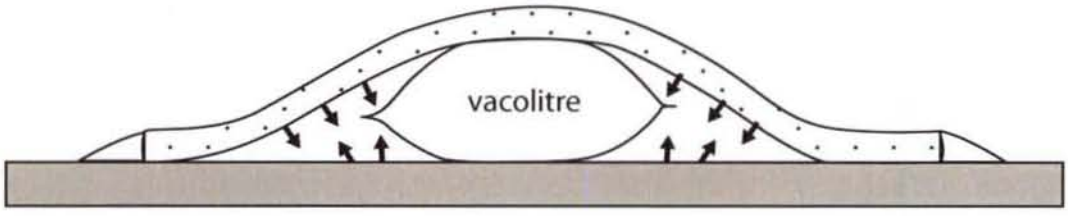

Fig. 8: Cross-sectional diagram of bag beneath non-circumferential NPWT, depicting the probable reason why the pressure increased within the bag. As it was occupying the space which would normally be obliterated when suction was applied, it was compressed between the foam and table, thereby undergoing an increase in pressure.

Had this been an ideal experiment, the measurement of the pressure being applied to the table would have been obtained without any object or sensor between the table and foam, and in so doing provide a more realistic account of whether the NPWT dressing applied an increased or reduced pressure to the underlying table. This would entail having a sensor flush with the table surface. Unfortunately there was no sensor available at that stage that would fit this description. The table experiments were therefore abandoned in search of a method whereby the sensor could be placed within the underlying substance, rather than on top of the substance. This would require that the substance on which the NPWT dressing was applied could be easily penetrated by the sensor. In addition, it would be required that it was reasonably compressible (unlike the table) to allow the sensor to record pressure changes. This inspired the "Plasticine experiments".

3.3.3. Plasticine experiments

Following the potential flaw in the methodology of the table experiments, Plasticine was used to test whether non-circumferential NPWT truly increases the pressure of the substance to which it is applied.

An intracranial tissue pressure microsensor (Codman/Johnson and Johnson Professional Inc., Raynham, Mass., USA), which makes use of a strain gauge transducer, was used to measure the pressure within the Plasticine (Fig. 9). It can be used to measure both positive and negative pressures in gas, liquids or any compliant substances, for example, soft tissue.

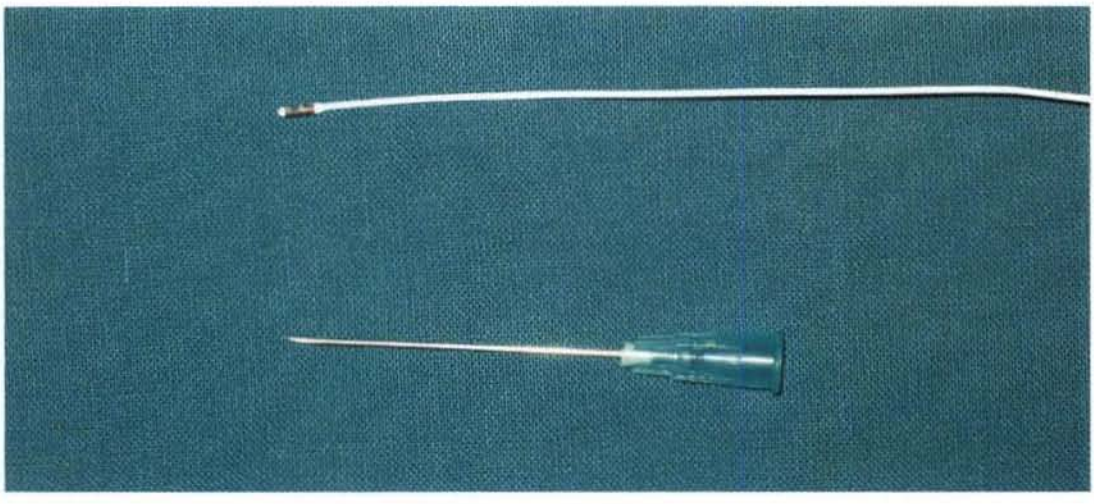

Fig. 9: Intracranial pressure microsensor, with standard 21-gauge needle for size comparison.

(A) Sensor in slab of Plasticine

The sensor was placed into the substance of a five millimetre- thick slab (10 x 6 cm) of Plasticine (using the introducer supplied by the manufacturer). Generic foam and a 3-mm diameter, perforated suction tube was used to complete a NPWT dressing on one side of the Plasticine slab (Fig. 10). Negative pressure was generated using a portable suction pump with an accurate pressure gauge (Schuco, Carle Place, N.Y., USA). After the transducer was zeroed, suction was applied to the dressing and the pressure change was recorded.

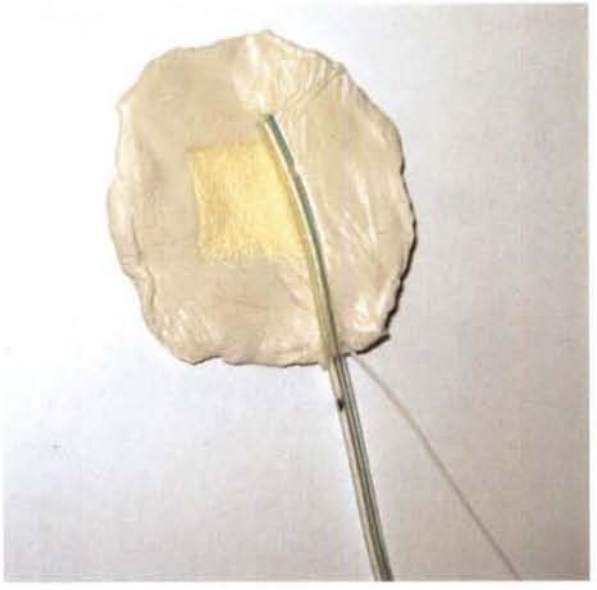

Fig. 10: Sensor in slab of Plasticine with NPWT in place.

It was found that the pressure within the plasticene increased for increasing suction pressures. This demonstrated that non-circumferential NPWT does, in fact, increase the underlying substance pressure. What caused this, however, was not known at this stage.

One theory was that the contracting foam and adhesive occlusive drape, which is attached to the substance, results in the substance being pulled toward the centre of the dressing, thereby compressing the substance beneath the dressing (in a horizontal manner). An alternative theory was that the atmospheric pressure outside of the dressing continues to apply pressure to the outside of the dressing in an attempt to equalise the pressure differential due to the hypobaric pressure within the dressing. As this equalisation can never be achieved because there is always a pressure differential created by the suction pump, significant forces on the outside of the dressing are created by the atmospheric pressure, which in turn, are translated to the underlying substance. The following experiments were devised to test this latter theory.

(B) Sensor between two slabs of Plasticine

To test the latter theory it was decided to place the NPWT dressing on one slab of Plasticine but instead of placing the sensor within the substance of the Plasticine it was placed between this slab and another one (Fig. 11). The hypothesis was that, if atmospheric pressure were to apply a downward force onto the dressing and, as a result, onto the Plasticine, then this Plasticine slab would, in turn, apply a force/pressure onto the sensor lying beneath it. If, however, the increased substance pressure were due to the substance of the Plasticine being drawn horizontally toward the centre point of the dressing, then this would have minimal, if any, effect on the sensor lying beneath the Plasticine slab.

Fig. 11: Sensor between two slabs of Plasticine with NPWT dressing in place.

On application of suction it was found that the pressure recorded by the sensor increased, implying that there appears to be a vertical (downward) force vector applied by atmospheric pressure onto the dressing, which may account for the increased tissue pressures observed on application of suction. This does not, however, imply that the latter theory (horizontal tissue compression as a result of the contracting foam) is not true, and in all likelihood both horizontal and vertical forces probably account for the increased tissue pressure observed during application of NPWT.

A new question arose, regarding the interface dressing. The fact that the interface dressing was occupying the space (between the adhesive occlusive drape and the underlying Plasticine) being obliterated by the suction force, would result in it being pushed into the Plasticine. Whether this was the cause for the increased substance pressure, or whether it would have increased even if there were no interface dressing pushing down on the Plasticine, was unknown. In other words, if a NPWT dressing was flush with the surface on which it is placed and there was no protruding part of the dressing (such as the interface dressing), which could be pushed into the underlying substance, would there still be an increase in substance pressure when suction is applied? The next experiment was undertaken In order to answer this question.

(C) Sensor in wall and base of cavity

A NPWT dressing can never be flush with a normal flat surface due to the fact that the interface occupies volume. For this reason, the interface dressing was placed within a cavity created in a block of Plasticine, so that the adhesive occlusive drape was flush with the surface (Fig. 12). Sensors were placed in two different areas; one was placed about 2 mm into the substance of the base of the cavity and the other 2 mm into the substance of the sidewall of the cavity. The sensors were introduced into the substance from an area away from the cavity. This avoided the possibility that the hypobaric air pressure from the NPWT dressing

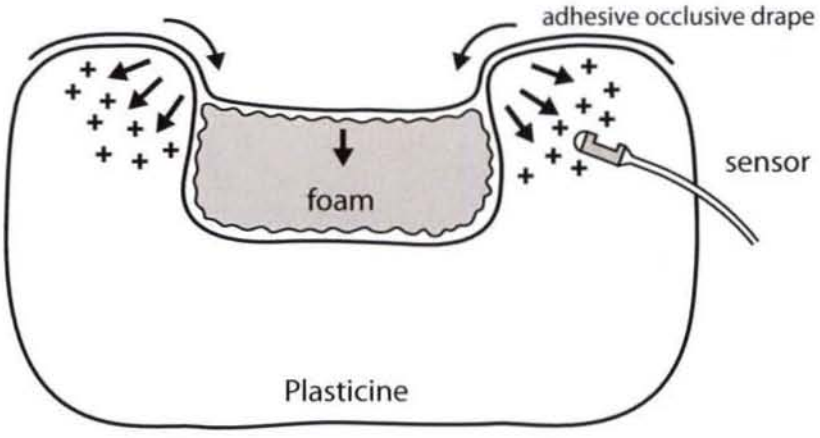

Fig. 13: Cross-sectional diagram of forces exerting pressure on outer surface of Plasticine surrounding cavity as foam collapses and draws with it the adhesive occlusive drape, which is attached to these surfaces.

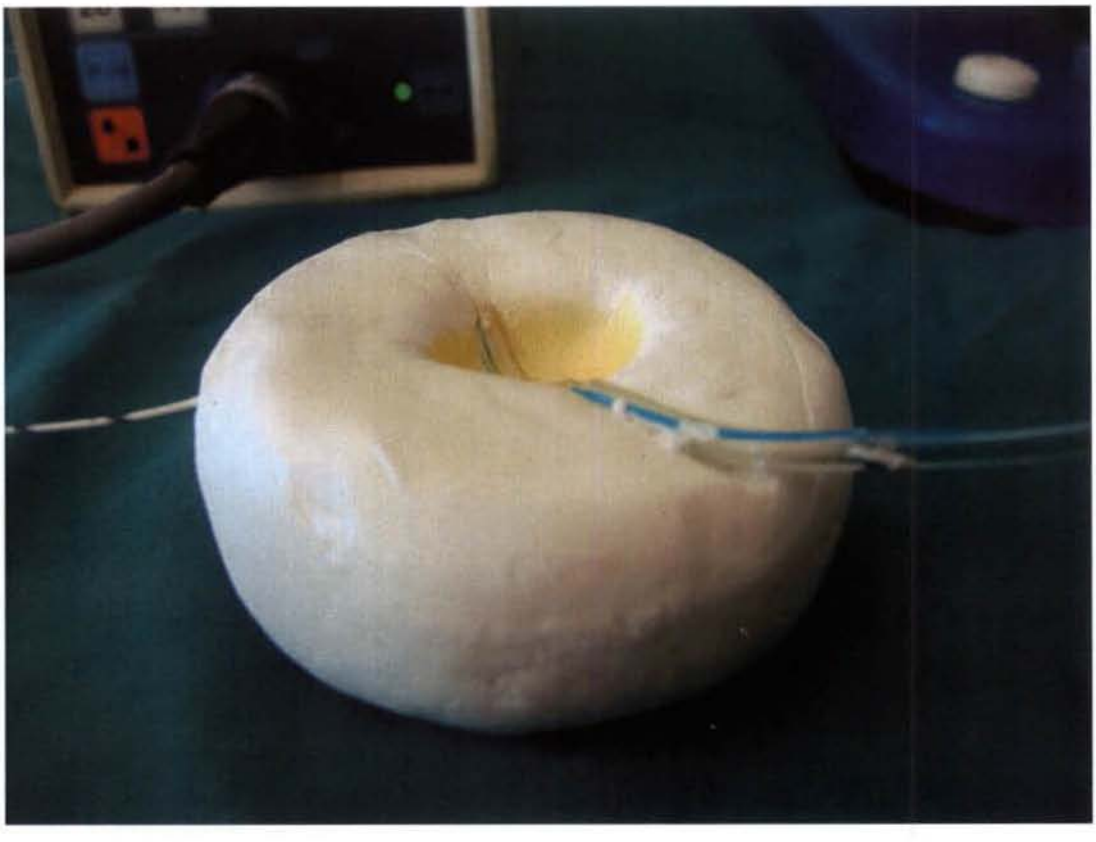

Fig. 14: Photograph illustrating the distortion of the edges of the cavity (compare to Fig. 12) as the adhesive occlusive drape applies a downward force due to the collapsing foam.

This did not, however, explain the pressure increase observed in the base of the cavity. A possible explanation for this could be that the walls of the cavity were drawn inwards toward the cavity, thereby compressing the substance in the base of the cavity (Fig. 15) and, in so doing, increase the substance pressure.

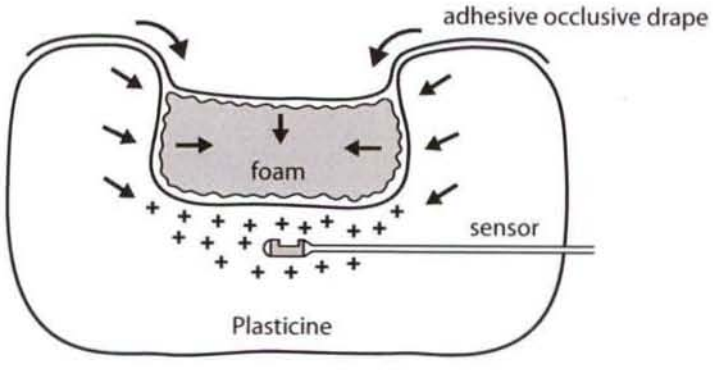

Fig. 15: Cross-sectional diagram illustrating the forces created by the walls of the cavity being drawn inward, resulting in the substance of the base being compressed, with a resultant increase in substance pressure.

(D) Air blown into NPWT dressing in cavity

The finding of this last experiment' was considered rather peculiar, as one would intuitively have expected that when foam collapsed inside a cavity the surrounding substance pressure would be reduced. As the opposite occurred and the collapsing foam increased the tissue pressure, the question arose as to what would happen in the case of expanding foam. Again, the intuitive answer would be that it should increase the pressure in the surrounding tissues.

In order to test this, an experiment identical to the previous one was carried out, but instead of air being sucked out of the suction tube, air was blown into the tube. Contrary to expectations, the substance pressure dropped to below baseline levels in both the base and walls of the cavity. This peculiar finding has yet to be explained but if the proposed explanations for the increase in tissue pressure during the previous experiment were correct, then presumably with the

force vectors reversed in this experiment, one can expect a reduction in substance pressure.

3.3.4. Banana experiments

The cavity Plasticine experiment illustrated the apparent importance of the adhesive occlusive drape's contribution to generating increased substance pressure. It was nevertheless difficult to believe that a sealed-off cavity which has hypobaric air pressure within it could result in increased surrounding tissue pressure.

With Plasticine, the area of adhesion of the adhesive occlusive drape is continuous and homogenous with the substance of the Plasticine. Placing a NPWT dressing in a cavity in a banana would create a different scenario. The banana can be seen as a container, with the area of adhesion of the adhesive occlusive drape being on the outside of the container (the skin of the banana), whilst the area experiencing the suction of the NPWT dressing is on the inside of this container. This setup would conceivably reduce the pressure within the container. This also more closely mimics the situation of a deep wound in a limb. An additional advantage of banana tissue is that it typically undergoes discolouration when exposed to pressure, thereby allowing a visual impression of pressure changes within the substance of the banana.

Therefore, after creating a cavity in the banana measuring 2 cm in diameter and 1 cm in depth, the same pressure sensors as in the previous experiments were placed at 1-cm intervals (three sensors) from the cavity. Care was taken to seal off the puncture hole around the sensor with Plasticine, to prevent air being sucked into the "container" during NPWT. The Plasticine also served to anchor the pressure sensors. In these experiments, both commercially available reticulated, open-cell foam (Kinetic Concepts, Inc., San Antonio, TX, USA) and generic foam were used as an interface dressing and care was taken to cut the foam flush with

the outer surface of the banana. The rest of the NPWT dressing was completed as in the previous experiments, with care being taken to place the suction tubing in the opposite direction to the pressure sensors. This was to avoid potential compression (by the suction tube) of those tissues being evaluated. (Fig. 16).

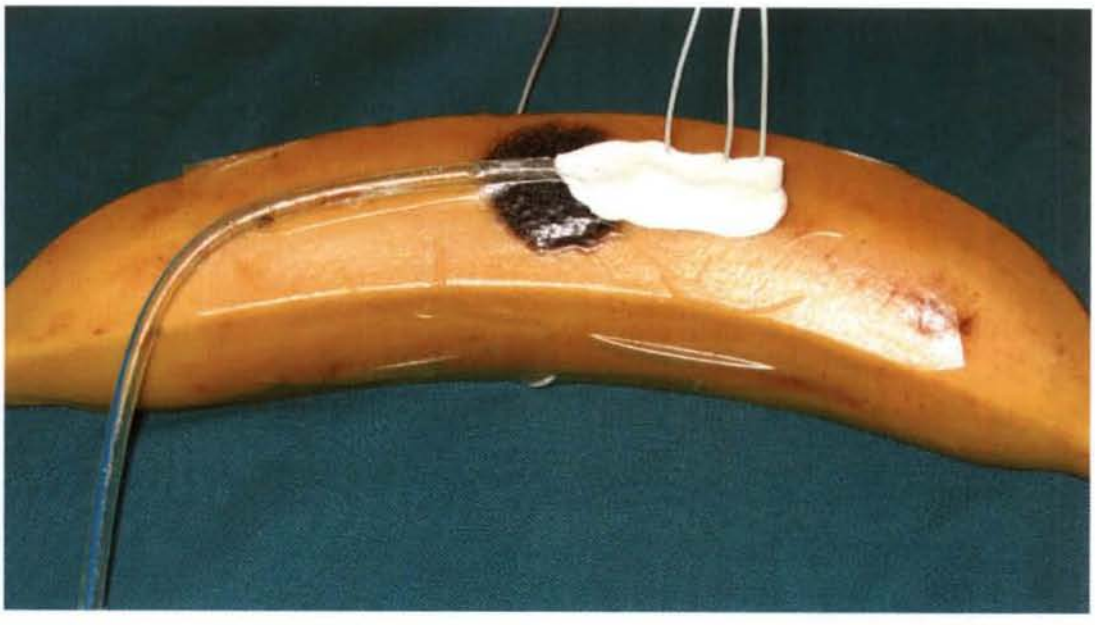

Fig. 16: Cavity in banana with commercial NPWT in place and pressure sensors at 1-cm intervals from dressing.

When suction was applied (-125 mmHg) the tissue pressure within 1 cm from the foam initially increased in pressure but after about one minute gradually returned to baseline, and occasionally even dropped to below baseline levels. The pressure further away from the cavity was largely unaffected, but occasionally reduced in response to suction.

Again, one could only speculate on the explanation for the above findings, which demonstrated initially increased pressure followed by a reduction in pressure (occasionally to below baseline). One explanation may be that, as in the Plasticine experiments, the compression elicited by the downward pull of the

adhesive occlusive drape served to increase the substance pressure adjacent to the cavity. As banana tissue tends to liquefy somewhat when compressed, this altered physical state may allow the tissue to be more susceptible to the suction forces within the dressing, rather than the compressive forces, thereby reducing the initially increased pressure. Indeed, some of this liquefied tissue could be seen in the suction tube.

Following completion of the experiment, the dressing was removed and the banana skin was removed to examine the flesh of the banana. A ring of discoloured tissue could be seen surrounding the cavity, corresponding to the areas which registered an initial increase in substance pressure (1 cm from cavity) (Fig. 17). The rest of the banana was not discoloured, except for the sensor puncture sites and an area that was compressed with a finger to ascertain the discolouration effects of external application of manual pressure.

Fig. 17: Flesh of banana demonstrating area of increased pressure surrounding cavity, corresponding to area initially recorded as having increased tissue pressure. The small circle of discolouration at the proximal end of the banana is due to a manual indentation, which was done to observe the effect that external force will have on the banana tissue. Sensor puncture sites are also discoloured.

The banana was then sliced at 1-cm intervals to obtain a cross-sectional perspective of the discolouration and, in so doing an impression of the depth to which the increased pressure penetrated. Again, it could be seen that the depth to which the pressure penetrated was greatest immediately beneath the cavity and less as the distance from the cavity increased (Fig. 18).

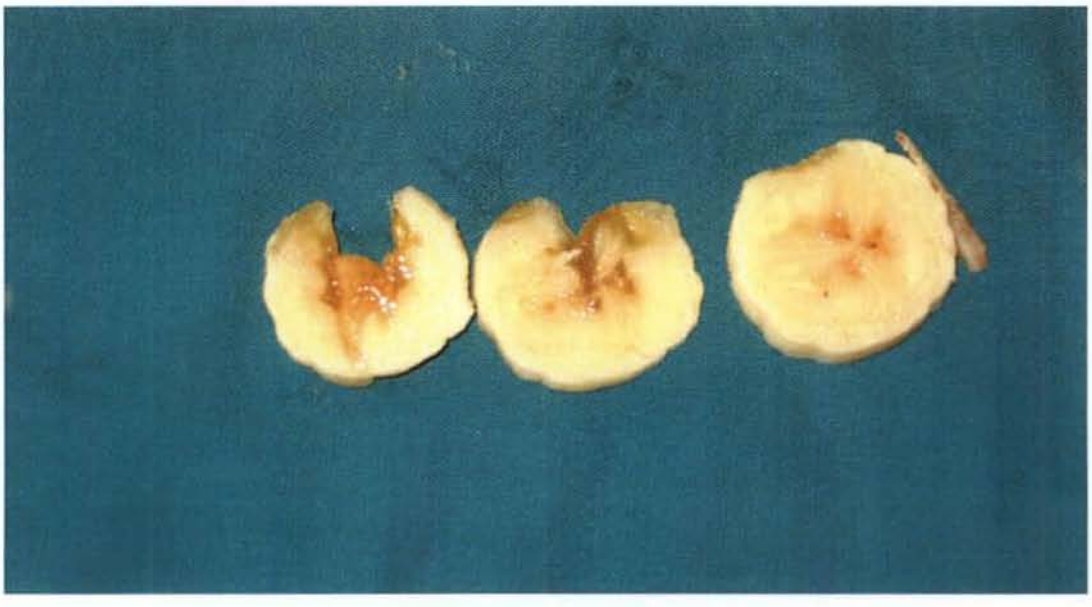

Fig. 18: Banana slices at 1-cm intervals from edge of cavity demonstrating the depth of penetration of the increased pressure being maximal close to the cavity (most proximal slice in photograph) and less as the distance from the cavity increased.

This was the first experiment to suggest that NPWT could result in hypobaric pressure, but possibly only if volume could be removed (in the form of fluid) from the substance. To test whether this postulate was true, a tissue with a greater volume of extractable fluid needed to be tested. Ideally the tissue-type should also represent a container, as in the banana experiment. For this purpose, an orange appeared to be the most appropriate subject, hence the next experiment.

3.3.5. Orange experiment

The NPWT dressing tends to apply compressive forces to the tissues, which can increase tissue pressure. However, the suction forces within the interface

dressing have the capability of rapidly removing fluid (if this fluid is amenable to extraction) and in so doing reduce the volume of the substance. If this substance is within a closed container (as in the relatively rigid outer skin of an orange), then this drop in volume is bound to result in a drop in tissue pressure.

This was tested by creating a 2-cm deep cavity with a 2-cm diameter in an orange and placing a NPWT dressing (using generic foam) within this cavity as per the previous experiments. The pressure sensor was introduced from the opposite side of the orange and lodged into a orange segment that was not in continuity with the cavity (an intact segment). The puncture site was sealed off with Plasticine to prevent air entering the site when suction is applied.

On application of suction (-125 mmHg), significant amounts of fluid was quickly extracted from the orange but the outer skin remained rigid and did not collapse under the hypobaric pressure (Fig. 19). The tissue pressure immediately became hypobaric and quickly reached the maximum negative pressure that the monitor could record (-99 mmHg).

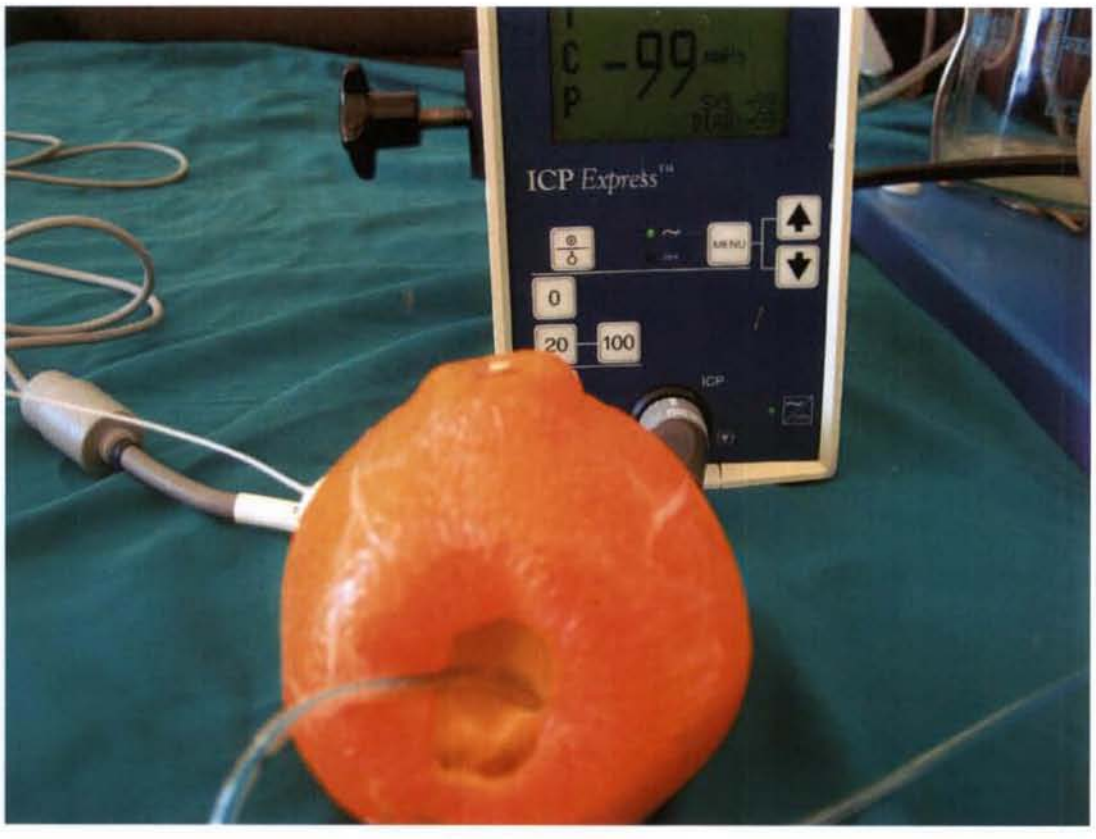

Fig. 19: NPWT dressing in 2-cm deep orange cavity during application of suction. Note orange skin retains its shape to a considerable degree, despite the significantly reduced pressure within the orange.

This experiment suggested that if a component of the substance undergoing NPWT is sufficiently fluid to be amenable to being extracted during NPWT (resulting in a reduction of the substance volume), then NPWT can result in significant hypobaric substance pressure. This is providing that this substance is within a relatively rigid container, which will resist collapse, as was the case in the orange.

If the container is allowed to change shape or reduce its capacity in response to the hypobaric pressure this is likely to affect the tissue pressure within the container. This raised an interesting hypothetical question, namely, if a container was allowed to reduce its capacity to zero during NPWT, compressing the contents within it, would the pressure of these contents be increased or reduced?

Put differently, was it possible to physically compress or crush a substance, without increasing the pressure of that substance? This gave rise to the "Syringe Experiments".

3.3.6. Syringe experiments

The earlier experiments already discussed gave the impression that there may be force vectors acting in two different planes, contributing to the increased tissue pressure seen in some NPWT experiments. One plane is horizontal, with the foam contraction resulting in the adhesive occlusive drape pulling the surrounding tissues toward the dressing. The other is in the vertical plane, as the pressure differential between the outside of the dressing and the inside of the dressing results in a downward force onto the dressing.

A syringe was seen as a potentially good model to mimic the latter force, the vertical (downward) force. During conventional NPWT it appears that the hypobaric pressure within the interface dressing causes the external atmospheric pressure to apply a force to the dressing's outer surface causing the foam to collapse. In a similar manner, if the inside of a syringe (with its plunger drawn out to its maximum) were to be exposed to hypobaric pressure, then the plunger would collapse down to obliterate this space.

Although this experiment did not cater for the horizontal forces likely to be prevalent in conventional NPWT, its purpose was not to mimic all the forces found in NPWT but to answer a basic physics question and, for this, the vertical collapse of the plunger was well-suited. The question to be answered revolved around the fact that, although the plunger is collapsing due to hypobaric pressure within the syringe, a substance placed within the syringe would be compressed by the plunger. Therefore it was unclear whether this substance would experience a pressure increase due to the compression or a decrease due to the ambient pressure within the syringe. The purpose of the syringe experiment was to answer

this specific question.

A 60-ml syringe was used. The plunger had a hole drilled into it to allow the placement of a suction tube through this hole. Silicone sealant was used to create an airtight seal between the drilled hole and the suction tube. Through the nozzle of the syringe, the pressure sensor used in the previous experiments was placed into whichever substance was to be tested and the nozzle was made airtight with Plasticine. In terms of comparing the syringe to the NPWT dressing, the syringe casing and the collapsible plunger represent the collapsible adhesive occlusive drape. Therefore, some squares of generic foam were placed inside the syringe to represent the interface dressing and also to prevent the suction tube becoming blocked by the substance to be compressed.

It was initially decided that the objects to be tested were slices of an orange, to see what pressure was registered within them despite their being crushed (Fig. 20). The sensor was place within one of these slices. A suction pressure of -125 mmHg was used. In this experiment pressure within the orange slices dropped to varying amounts of hypobaric pressure, despite their being totally deformed by the compressive forces of the plunger. The amount of hypobaric pressure seemed to be counterbalanced by an increasing amount of positive pressure (which gradually brought the measurements closer to atmospheric pressure) as the orange slices were more compressed.

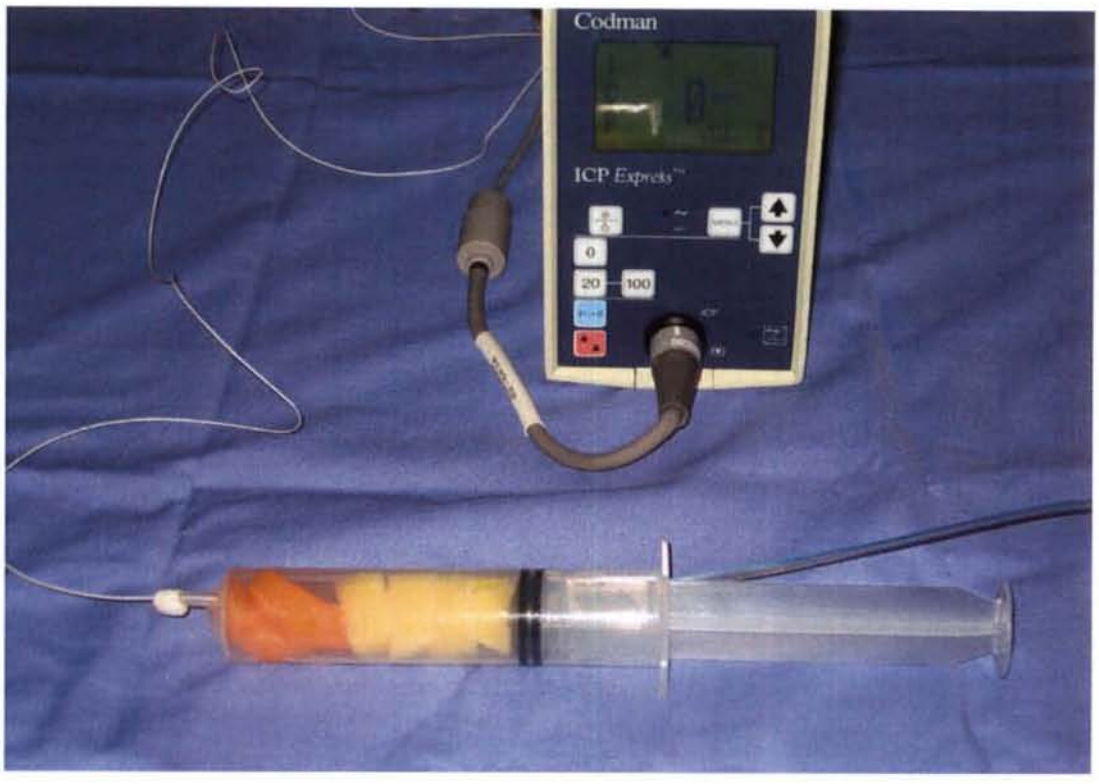

Fig. 20: Syringe experiment setup with orange slices about to be compressed by plunger when suction is applied.

A potential flaw was seen in the design of this study design, in that when the orange was deformed by the plunger, the sensor may have been pushed through the substance of the orange slice, possibly coming into contact with the hypobaric air pressure within the syringe. The results in that case would therefore merely represent the pressure within the syringe and not necessarily the pressure within the substance of the compressed orange slice.

Consequently, a second experiment was done, this time using a cube of processed meat as the substance to be compressed (with the sensor within this substance). This experiment was repeated five times, and again, maximal hypobaric pressures were recorded in the substance of the meat despite the plunger pushing down against it.

This demonstrated that it is possible physically to compress and deform a substance, yet keep the pressure hypobaric within the substance. This appears peculiar and there may be inherent reasons for why this occurred in this specific experiment, such as the existence of a large amount of friction between the plunger and the syringe walls, which prevents the plunger from applying the maximal amount of force on the substance. If there was no friction and the plunger was allowed to move freely, then the equalising forces of atmospheric pressure may have applied enough pressure to increase the substance pressure. Therefore, although this experiment answered the physics question of whether it is possible to compress a substance and retain hypobaric pressure within it, this experiment's findings cannot necessarily be extrapolated to what might occur during NPWT *in vivo*.

3.3.7. Chicken meat in vitro *experiment*

Although evidence was mounting to support the fact that NPWT increases the pressure within the substance to which it is applied, depending on the type of subtance, it was necessary to see whether the same would occur in animal tissues. A large breast of chicken meat, complete with skin, was procured to test this.

A small, 2-cm diameter, shallow defect was created in the skin and underlying muscle (Fig. 21). The pressure sensor used previously was placed 5 mm deep to this "wound" and generic foam was used to create a NPWT dressing. Suction was applied at -75 mmHg, -125 mmHg and -400 mmHg. On application of suction (at all the suction pressures tested), there was no change in pressure recorded within the meat.

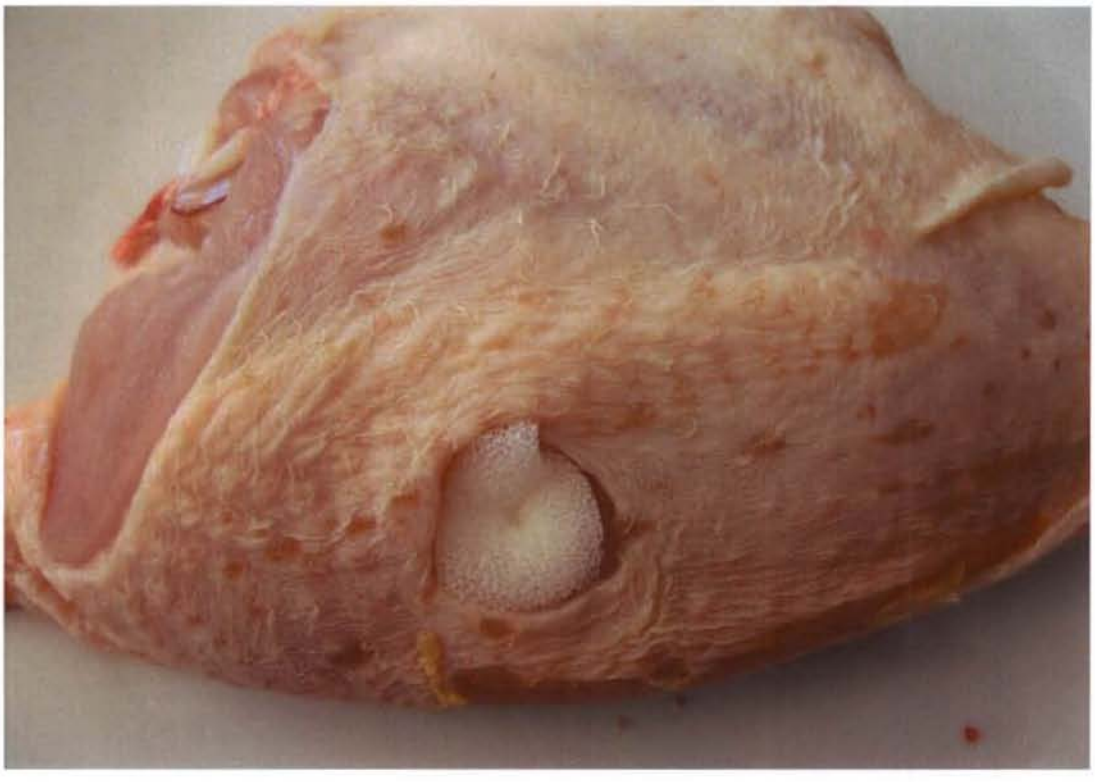

Fig. 21: Chicken breast with skin and shallow "wound" within which a NPWT dressing was to be placed using generic foam. Note the laxity of the skin in the upper right of the breast.

A potential reason for this may have been that the chicken skin was extremely mobile. When the foam contracted, the adhesive occlusive drape and attached chicken skin could be drawn toward the wound without drawing the underlying muscle in this direction. It was therefore unlikely that there was any significant horizontal compression of this muscle tissue to result in a recordable change in pressure. In addition, the previously mentioned vertical force vectors appeared to be insignificant in this experiment. Furthermore, as mentioned earlier, during foam contraction the adhesive occlusive drape and attached skin moves toward the wound. In a real wound this would serve to tighten the skin and in so doing, constrict the limb (to a degree). This in turn, may cause an increase in tissue pressure. However, if the skin does not circumscribe the limb (or piece of chicken in this case) then no such constriction occurs. This could be another reason for the lack of tissue pressure increase in the chicken experiment.

Although the excessive skin mobility was thought to be one of the causes of the absence of a reading (resulting in this model being abandoned), it may have illustrated that the attachment of the skin to the underlying tissues is an important variable in how much pressure is generated within tissues. The more loosely attached the skin is, the smaller the increased pressure generated within the tissues. This may also have illustrated the significance of the force vectors which occur in the lateral plane. It appeared that the elimination of these horizontal vectors, due to the chicken skin being loosely attached to the muscle (thereby not transferring these horizontal forces to the muscle), resulted in an absence of any recordable pressure change within this muscle.

3.3.8. Pork meat in vitro *experiment*

The effect of NPWT on animal tissue was not adequately answered by the chicken breast experiment, with one possible reason being the lax skin (the other being that the skin didn't circumscribe the tissue). The skin of pork, on the other hand, is more tightly adherent to the underlying muscle and therefore a large pork loin was procured for this experiment.

(A) Sensor above skin

An initial experiment was conducted (again using generic foam), whereby the sensor was placed directly beneath the foam, on top of intact skin. On application of suction at -125 mmHg, the sensor recorded its maximal limit of hypobaric pressure (-99 mmHg). This was expected due to the sensor being in continuity with the foam's air pressure.

It must be noted at this stage that for the sensor to successfully record pressure within a substance, the substance must be able to apply pressure to the microscopic strain gauge of the sensor. This strain gauge is housed within a recessed window at the sensor tip. Therefore this sensor cannot, for example, measure pressure when a rigid object is pushed against the rigid sensor tip, as

this would not apply direct pressure to the recessed strain gauge. Although the foam is not rigid, it is unlikely to conform sufficiently into this recessed window to apply force to the strain gauge. The ambient air pressure, however, can apply such forces to the strain gauge and it was for this reason that it could be expected to see the sensor recording hypobaric pressure. An interesting observation in this experiment was the indentation that the sensor, suction tube and foam left on the skin after removal of the dressing, illustrating the vertical force that was applied to the tissues (Fig. 22). Another interesting observation was the fact that, even in this pork model, the skin was pulled toward the dressing to some degree by the adhesive occlusive drape, as the foam contracted (Fig. 22).

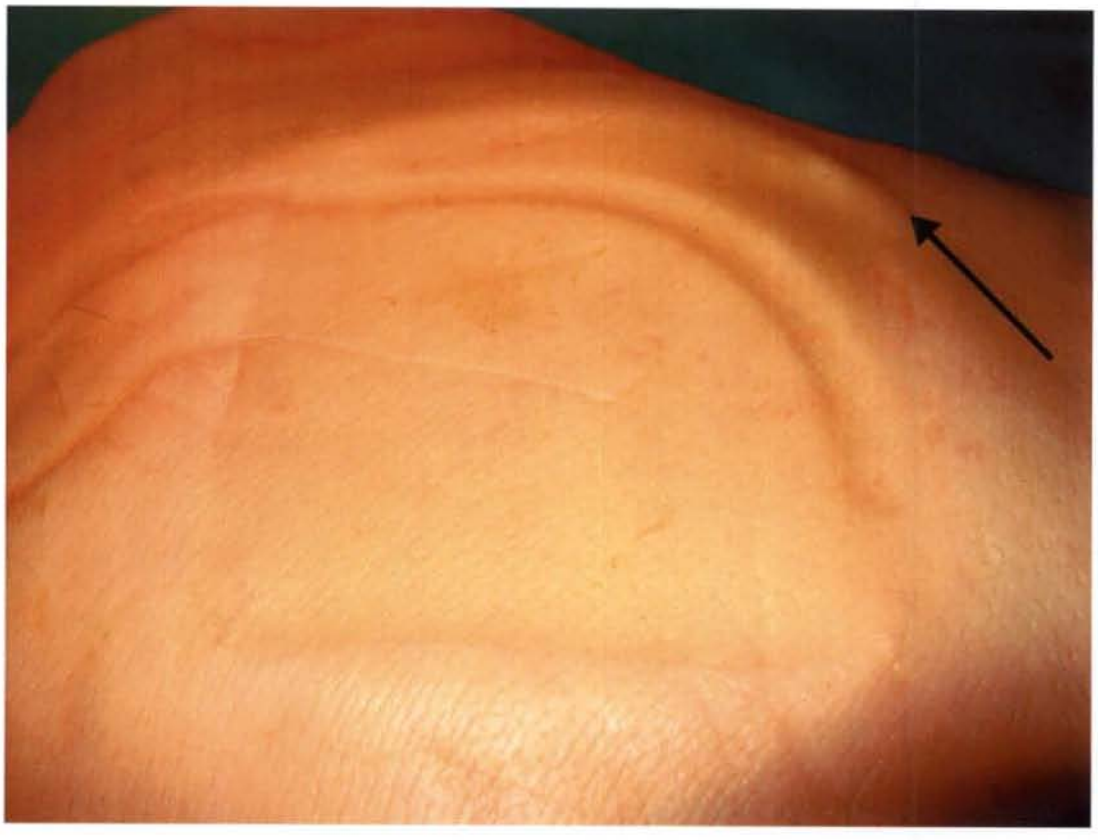

Fig. 22: Impressions left on pork skin by sensor, foam and suction tube (despite tube being laid between two slabs of foam), indicating downward force (vertical force) applied by NPWT dressing. Notice the skin fold (arrow) due to the skin being pulled toward the dressing during foam contraction, indicating the additional horizontal forces that occur.

(B) Sensor below skin

A second experiment was carried out, this time placing the sensor in the muscle beneath the skin (via the cut end of the meat). The same NPWT dressing was placed, ensuring that the dressing was above the sensor. On application of suction at -125 mmHg, there was only a small increase in tissue pressure (2 mmHg). Again, this inconspicuous increase may have been because the skin was more mobile on this cut leg of pork than what would be the case *in vivo*. Probably more important, however, was the fact that the skin did not circumscribe the muscles in which the sensor was placed. When they were drawn inwards toward the wound, they did little to compress the underlying muscle tissue.

From these experiments it appeared that animal tissue exposed to NPWT was hyperbaric, albeit minimally so. Even higher tissue pressures may have been recorded were it not for the laxity of the skin and the fact that it did not circumscribe the meat.

3.3.9. Head ring gel experiments

The first part of the previous experiment highlighted the importance of the sensor's recessed strain gauge being in contact with the substance being measured. This raised the question of how representative the reading is of the tissue pressure, if there is the possibility that it may not be in contact with the substance being measured (Fig. 23). This would be more relevant in the experiments which recorded hypobaric tissue pressure, despite substances being compressed, for example in the banana experiment.

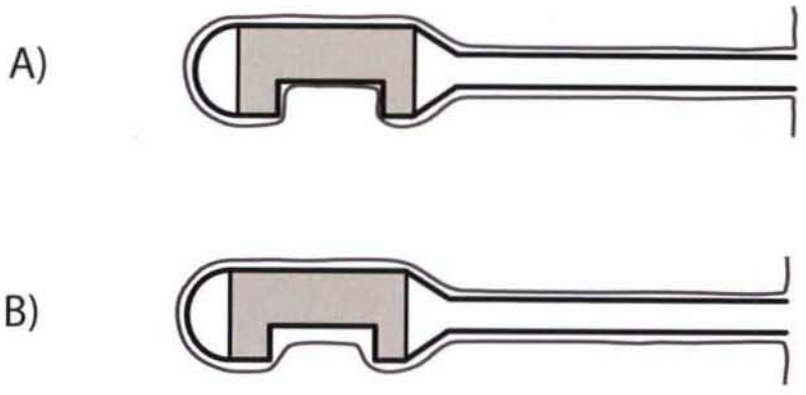

Fig. 23: Illustration depicting the potential for the sensor to be recording the pressure of a small pocket of hypobaric air in the recessed window of the strain gauge. (A) At initial compression, the tissues may be in contact with the strain gauge. (B) After distortion of the tissue due to compression or removal of some fluid, the tissue may move away from and lose contact with the strain gauge, thereby creating a small pocket of hypobaric air in front of the strain gauge. The sensor would then record hypobaric pressure, despite the pressure within the tissues being compressed being hyperbaric.

For this reason it was decided to conduct a NPWT experiment and test the pressure of a substance which was likely to stay in contact with the strain gauge. The substance had to be extremely conformable, to allow filling of the recessed window, but not fluid enough to allow drainage of the substance. The semi-solid, soft, gel-like substance found in "head rings", which are used to prevent scalp pressure sores during surgery, was deemed an appropriate substance for this purpose (Fig. 24).

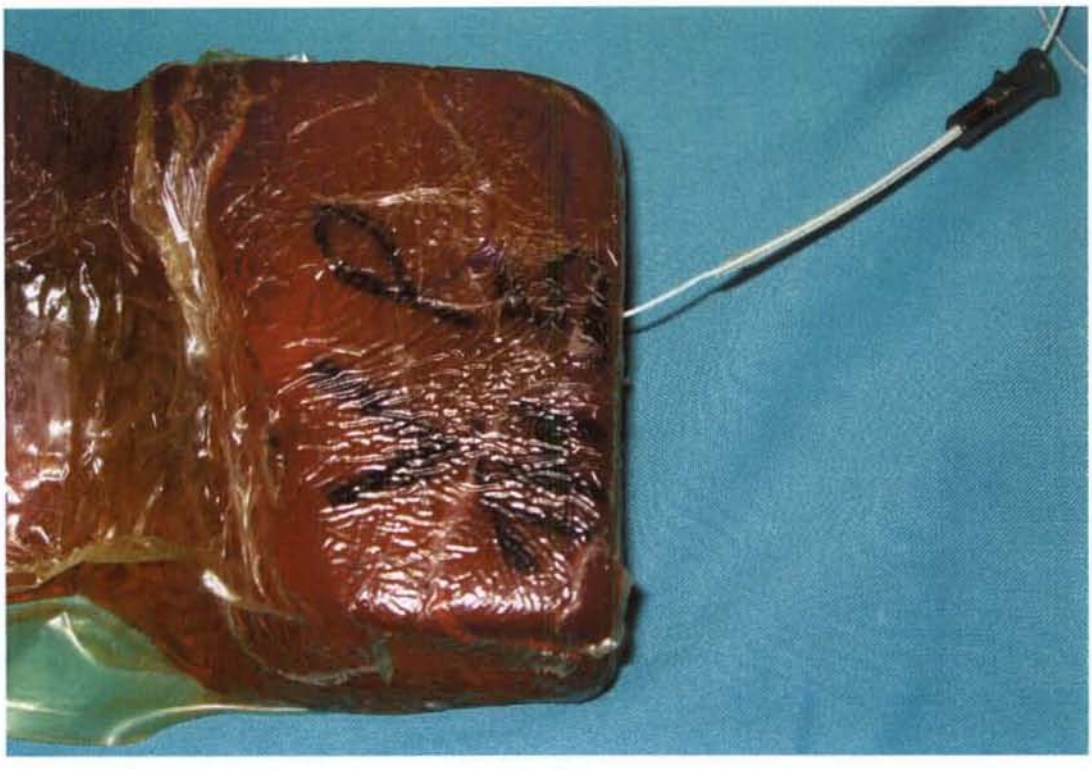

Fig. 24: Head ring, with pressure sensor placed into the gel-like substance.

After placing the sensor into the substance using an introducer, and zeroing the sensor, the substance was first manually compressed by applying a downward force, to ensure that this would result in increased tissue pressure. Contrary to expectations, the pressure within the substance became hypobaric. This manual compression was repeated numerous times and yielded the same result.

This substance may have proven itself to be the least appropriate substance for this experiment. It was noted that the gel expanded laterally during the manual application of downward force, raising the question of whether the hypobaric pressure was caused by the strained gel moving out of the recessed window, as it bulged out laterally. It was therefore speculated that the reduced pressure recorded may, in fact, have been due to the gel substance moving away from the recessed strain gauge, as the tension in the substance increased. This, in fact, would result in the scenario depicted in Fig. 23 (B) rather than the expected

scenario depicted in Fig. 23 (A).

When the manual compressive force was applied from two opposing directions, it too resulted in hypobaric pressure, as the gel bulged out laterally. However, when the force was applied from all directions by compressing it in cupped hands, it was possible to increase the pressure within the gel. By preventing the gel from bulging laterally by applying forces from all directions, the gel would be forced to bulge into the recessed window of the strain gauge, thereby applying a force to it and resulting in the recording of a positive pressure (as depicted in Fig. 23 (A))

Following the manual pressure tests, a NPWT dressing using commercially available reticulated, open-cell foam (Kinetic Concepts, Inc., San Antonio, TX, USA) as an interface dressing was applied to the surface of the gel pad. On application of suction, there were no noticeable changes in pressure within the gel substance. One such experiment (using a flatter type of pressure sore preventing gel) again revealed the presence of the horizontal forces which are applied to tissues. The contraction of the foam and the pull of the adhesive occlusive drape resulted in the gel pad curling upward at its edges, as the drape pulled on the superficial surface of the pad (Fig. 25).

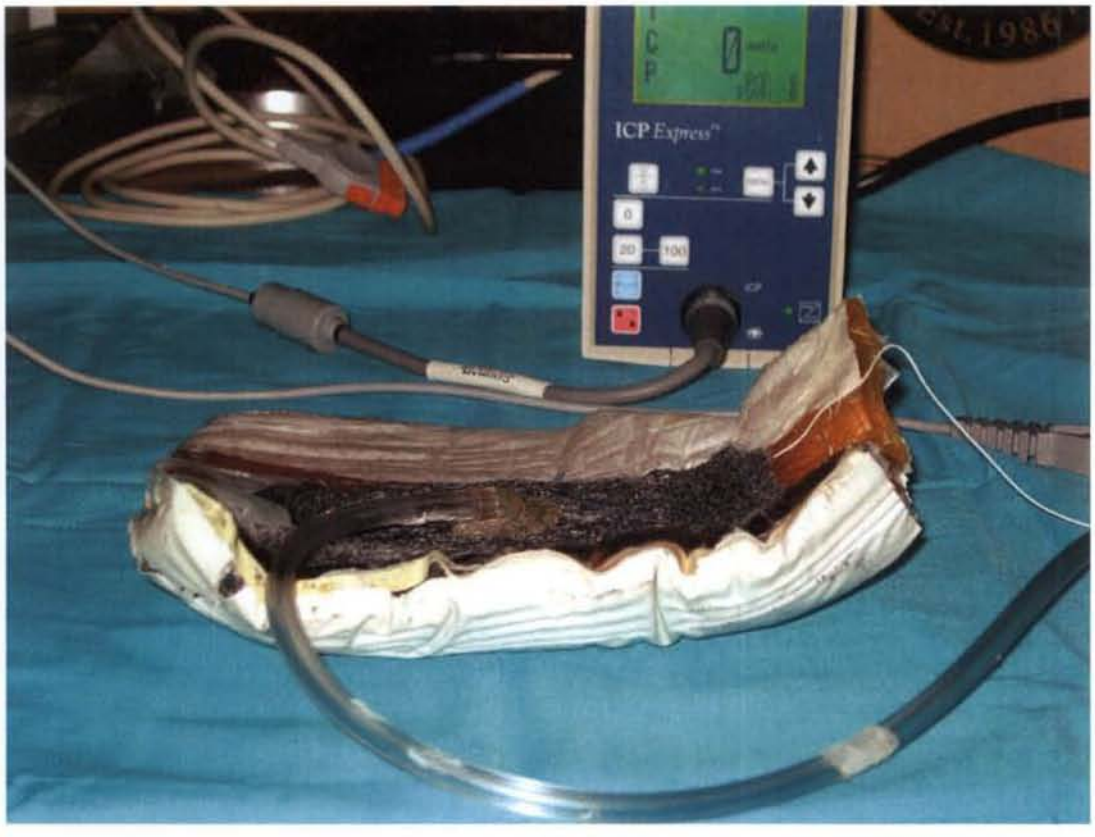

Fig. 25: Flatter gel pad with NPWT dressing with suction applied. Notice the edges of the pad curling up due to the horizontal forces of the NPWT dressing as the foam contracts.

The gel experiments did not provide any factual evidence regarding NPWT and tissue pressure. They did, however, appear to demonstrate that the recording of tissue pressure is more complicated than initially thought.

3.3.10. Jelly experiments

The gel experiments prompted further thought on attempting to ensure that the pressure sensor is indeed recording the pressure within the substance undergoing NPWT. The only way to ensure that the strain gauge of the sensor was in total contact with the substance would be if the substance was fluid enough to completely fill the recessed window within which the strain gauge is housed. It would be impossible to apply NPWT to such a fluid substance, however, as this would immediately be drained away. This was unless the state of the substance could be changed from a liquid to a more solid form, *after* the sensor was placed.

This is what inspired the jelly experiment.

(A) Tub-of-jelly experiments

Two sensors were placed into a 10x10x3-cm tub of liquid jelly. One positioned to be directly beneath the NPWT dressing at a depth of 1 cm and the other placed at a distance of 1 cm away from the NPWT dressing, also at a depth of 1 cm. The liquid jelly was refrigerated to allow the jelly to set, in order to allow the application of NPWT. The tub was removed from the refrigerator after 24 hours and a generic-foam NPWT dressing was placed, with the adhesive occlusive drape sticking to the sides of the tub (Fig. 26) Application of suction at -125 mmHg resulted in a near-identical hypobaric pressure recording in both sensors within the jelly (± -30 mmHg).

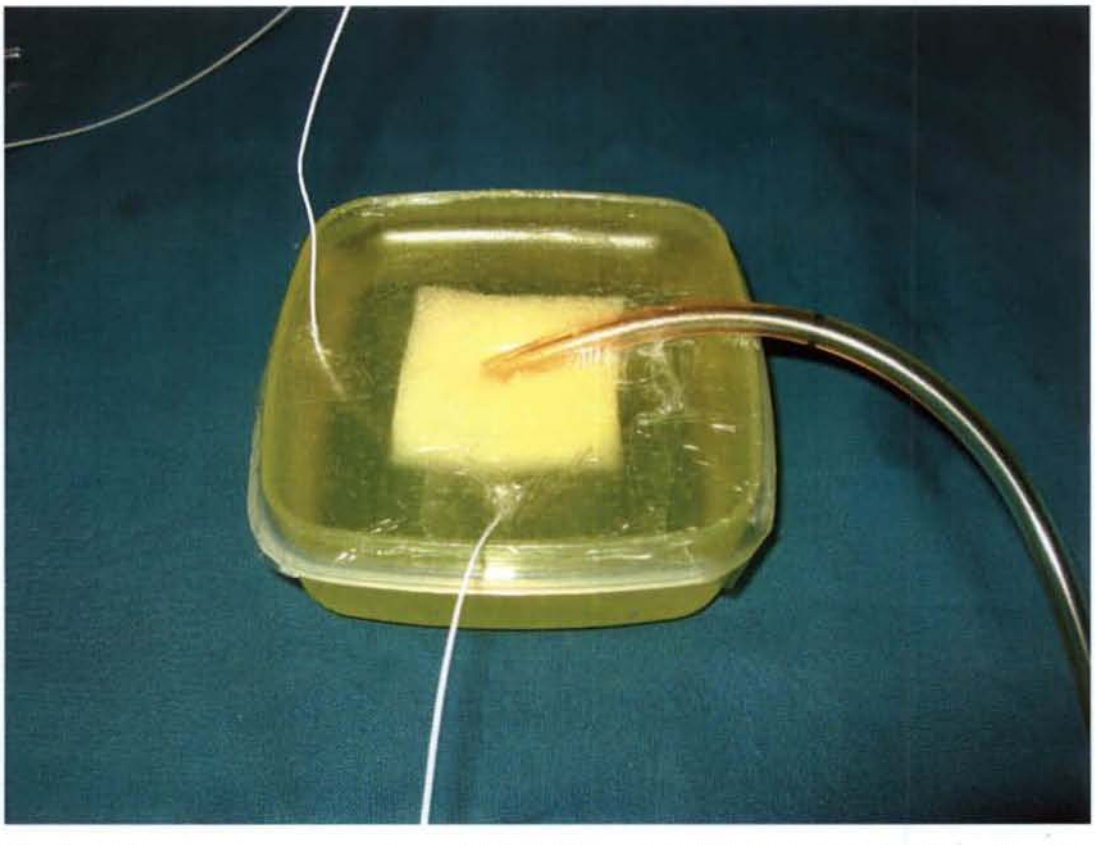

Fig. 26: Two pressure sensors in a set tub of jelly with a NPWT dressing in place. Note the adhesive occlusive drape is attached to the edges of the tub too.

It was noted during this experiment that the attachment of the adhesive occlusive drape to the sides of the rigid tub may have resulted in the NPWT dressing being prohibited in its application of compressive forces, both vertically and horizontally, hence the resultant hypobaric pressure within the jelly (this was quite similar to applying negative-pressure to a substance within a rigid container).

An identical experiment was therefore conducted but this time the jelly was removed from the tub. After the surface of the jelly was damped dry (to allow the adhesive occlusive drape to stick) an identical NPWT dressing was placed. Application of suction in this model resulted in hyperbaric pressure being recorded. The sensor beneath the dressing recorded approximately 7 mmHg, while the one lateral to the dressing recorded about 2 mmHg (Fig. 27).

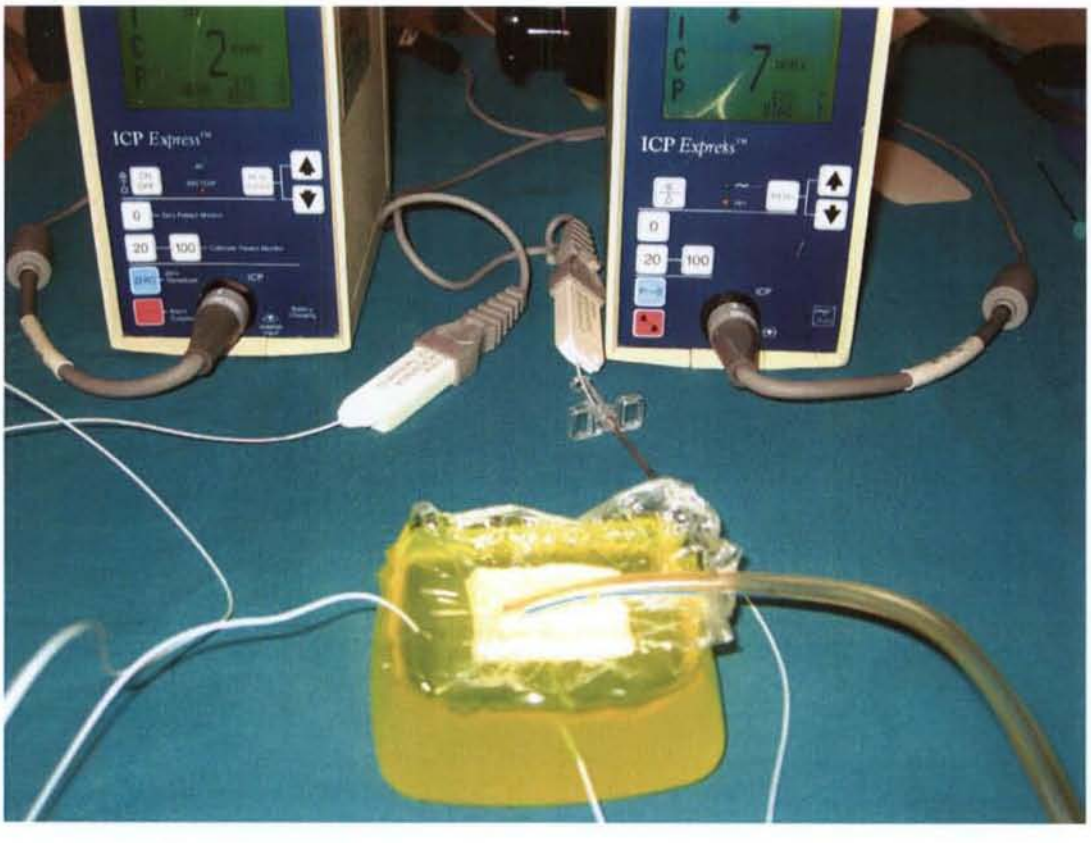

Fig. 27: Jelly after having been removed from tub with sensors placed beneath and at a distance of 1 cm from the NPWT dressing.

(B) Tray-of-jelly experiments

A similar experiment was conducted to the first experiment in the Jelly Experiment range but instead of a small tub, a large tray was used. The objective was to evaluate pressure changes at different depths beneath the NPWT dressing and also at different distances from the NPWT dressing.

A total of five sensors (identical to those used in the previous experiments) were used and fixed to the bottom of the tray using Plasticine. Two were intended to be directly beneath the NPWT dressing but at different depths (one at a depth of 1 cm and the other at a depth of 3 cm). The other three were placed so that they would be 2 cm, 4 cm and 6 cm from the dressing, at a depth of 1 cm below the surface (Fig. 28).

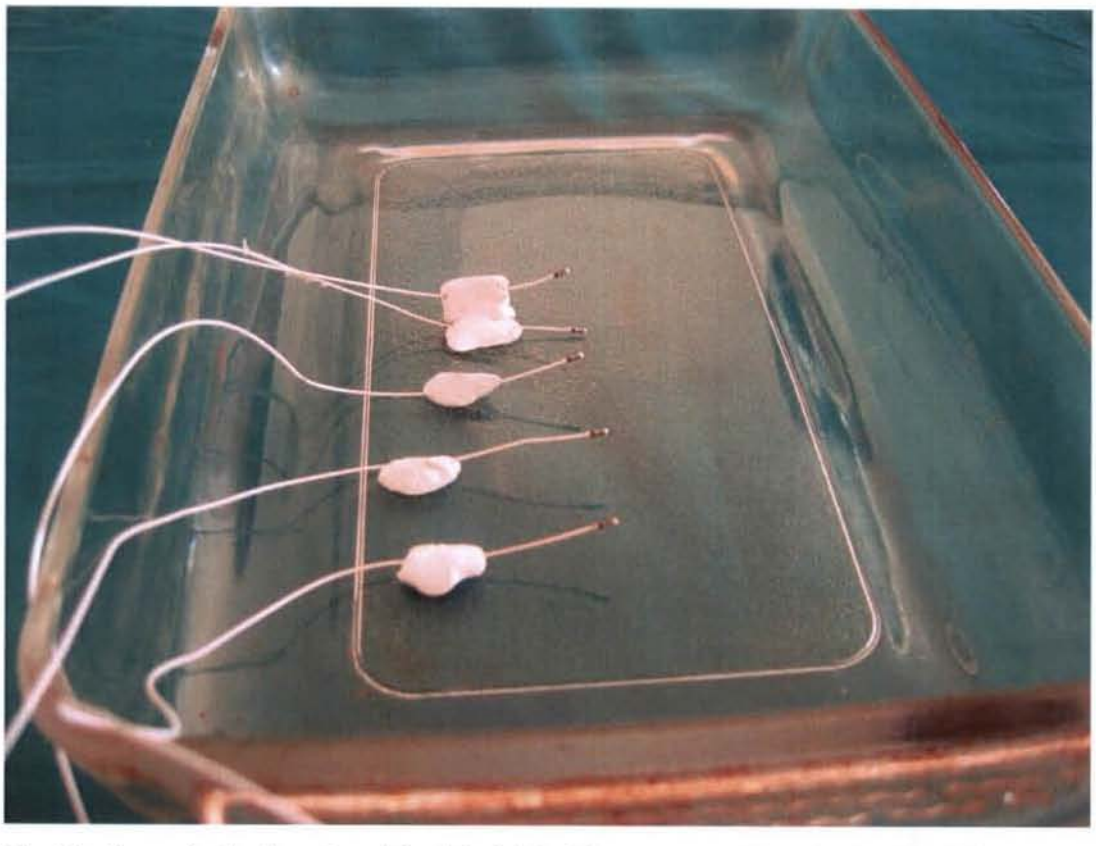

Fig. 28: Tray prior the insertion of the jelly fluid, with sensors positioned using Plasticine.

The liquid jelly was poured into the tray so that the sensors were covered at the appropriate depths and allowed to set over 24 hours in a refrigerator. Following this, a NPWT dressing using generic foam (5x5 cm) was positioned as planned. The jelly was not removed from the tray, for fear of it fracturing. However, the adhesive occlusive drape was not allowed to touch the sides of the container, thereby allowing both vertical and horizontal forces to be generated. Manual pressure applied to the dressing confirmed that the pressure recorded by the sensors beneath the dressing was hyperbaric (unlike in the "head ring gel experiments"). The other sensors lateral to the dressing did not record a change during manual pressure application over the dressing.

On application of suction at -125 mmHg, there was only a slight increase in the jelly pressure (2 mmHg at a depth of 1 cm and 1 mmHg at a depth of 3 cm). The jelly pressure in the other sensors fluctuated between -1 mmHg and 0 mmHg and did not seem to settle on a single value. The effect of distance from the dressing was therefore difficult to ascertain. The reliability of this experiment, however, was drawn into question because on application of suction the horizontal contractile forces resulted in the jelly fracturing in some areas, which was likely to have changed the compressive forces that would have been applied to the jelly (Fig. 29).

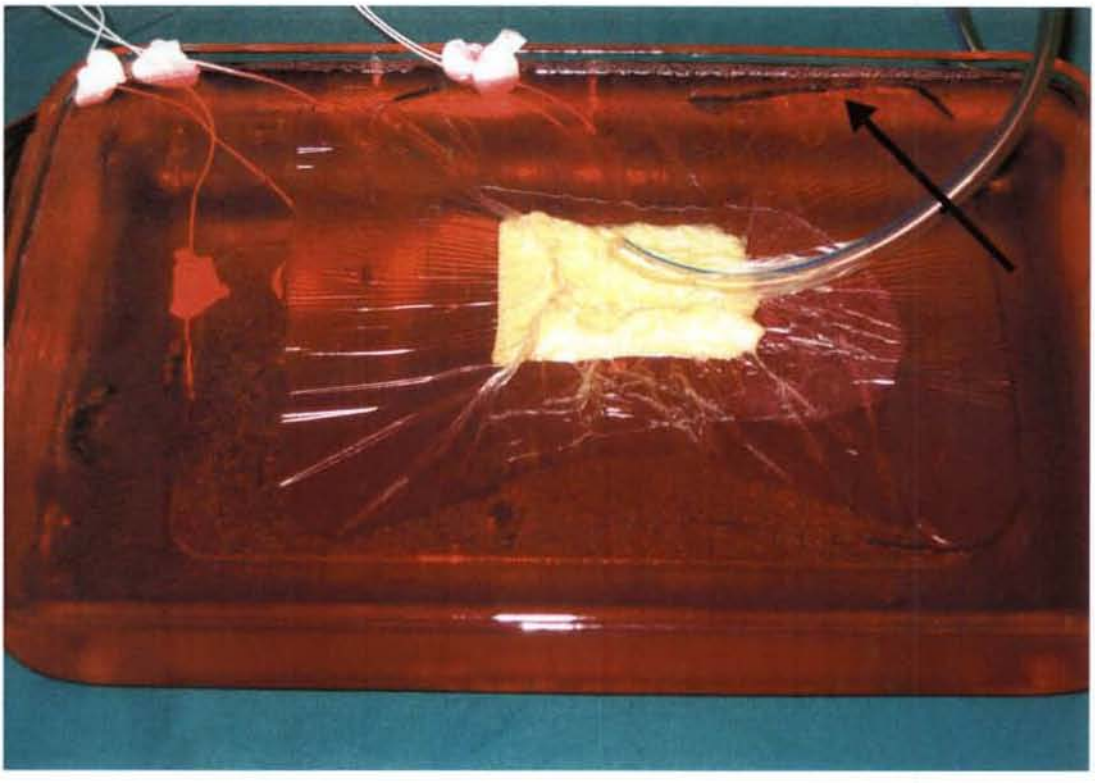

Fig. 29: NPWT dressing with suction being applied. Note fracturing of the jelly due to the horizontal contractile forces applied to the jelly (arrow).

The latter jelly experiment again confirmed the presence of the previously mentioned horizontal forces that are being applied to the underlying substance. The jelly experiments also confirmed that having a substance in total contact with the strain gauge is important. However, the fragile nature of jelly made this a poor model to be used for further research. The reason these sensors are reliably used in human tissues, is that there is always an amount of fluid around the sensor in living tissues (unlike the case of the head ring gel). This fluid can easily occupy the entire recessed window and thereby accurately transfer the pressure to the strain gauge.

3.3.11. Water-in-glove experiment

Despite the number of experiments conducted thus far, there was still a poor understanding of exactly what caused the increase in substance pressure during

application of NPWT, especially regarding the vertical forces. One theory regarding the origin of the vertical force was that atmospheric pressure would continue to bear down on the dressing until the (hypobaric) space between the adhesive occlusive drape and the underlying substance was obliterated. However, this space could never be obliterated due to the occupation thereof by the interface dressing. This interface dressing would therefore be partially displaced into the substance by the downward force, which in turn, would result in an increase in substance pressure.

However, as the spaces in the interface dressing (pores in the case of foam) are never totally obliterated, and always remain hypobaric due to the constant suction which is applied, a pressure differential will always remain on either side of the adhesive occlusive drape. The implications of having a persistent atmospheric pressure differential were not known. There were two theories of what might have occurred. In the one, it was hypothesised that atmospheric pressure would continue to apply an unabated force regardless of how deep the interface dressing was displaced into the substance. In the other it was hypothesised that this force would stop once the adhesive occlusive drape was relatively flush with the rest of the substance (when the interface dressing had reached maximal collapse it would not push into the substance any further).

In order to answer this, a substance was required (on which a NPWT dressing could be placed) that would allow minimal resistance to the downward displacement of the interface dressing (generic foam in this case). If the former theory was correct, the NPWT dressing would continue to displace the substance so long as suction was maintained. If the latter theory was correct, the substance would only be displaced until the adhesive occlusive drape was relatively flush with the surface of the substance.

A surgical glove filled with water was used. Applying a force to the surface of this glove resulted in the surface being displaced nearly effortlessly and a finger of the glove could be manually flattened with hardly any force. Two experiments were conducted.

(A) NPWT to hand and fingers of glove

In this experiment, a sandwich-type NPWT dressing (generic foam) was placed over the hand and fingers of the glove. If the former theory was true (atmospheric pressure would apply an unabated force) then the fluid within the glove between the slabs of foam would be displaced to the portion of the glove which was not between the foam, resulting in this portion bulging out. If the latter theory were true, then the foam would collapse (as completely as possible) onto the glove surface and then push no further into the glove, and therefore not cause any fluid displacement (Fig. 30). On application of suction at -125 mmHg it could be seen that there was no noticeable displacement of fluid and no bulging of the glove outside of the NPWT dressing (Fig. 31). Even the fingers remained fully inflated with water.

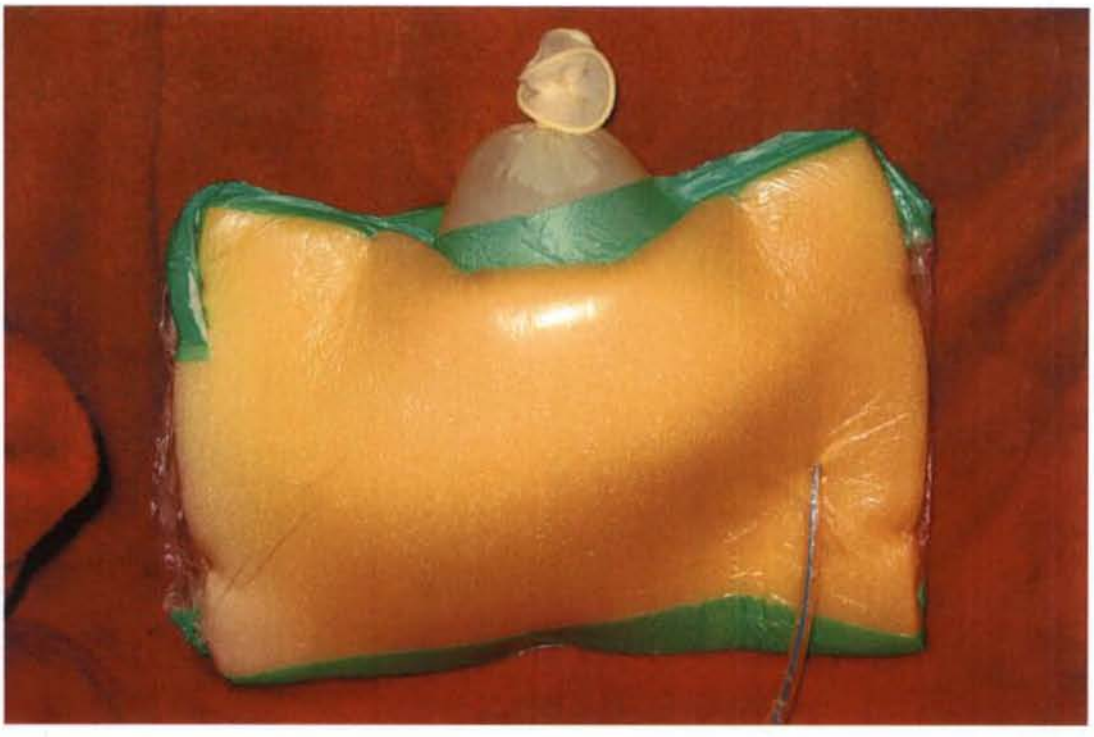

Fig. 30: Sandwich NPWT dressing applied to glove filled with water, without suction applied.

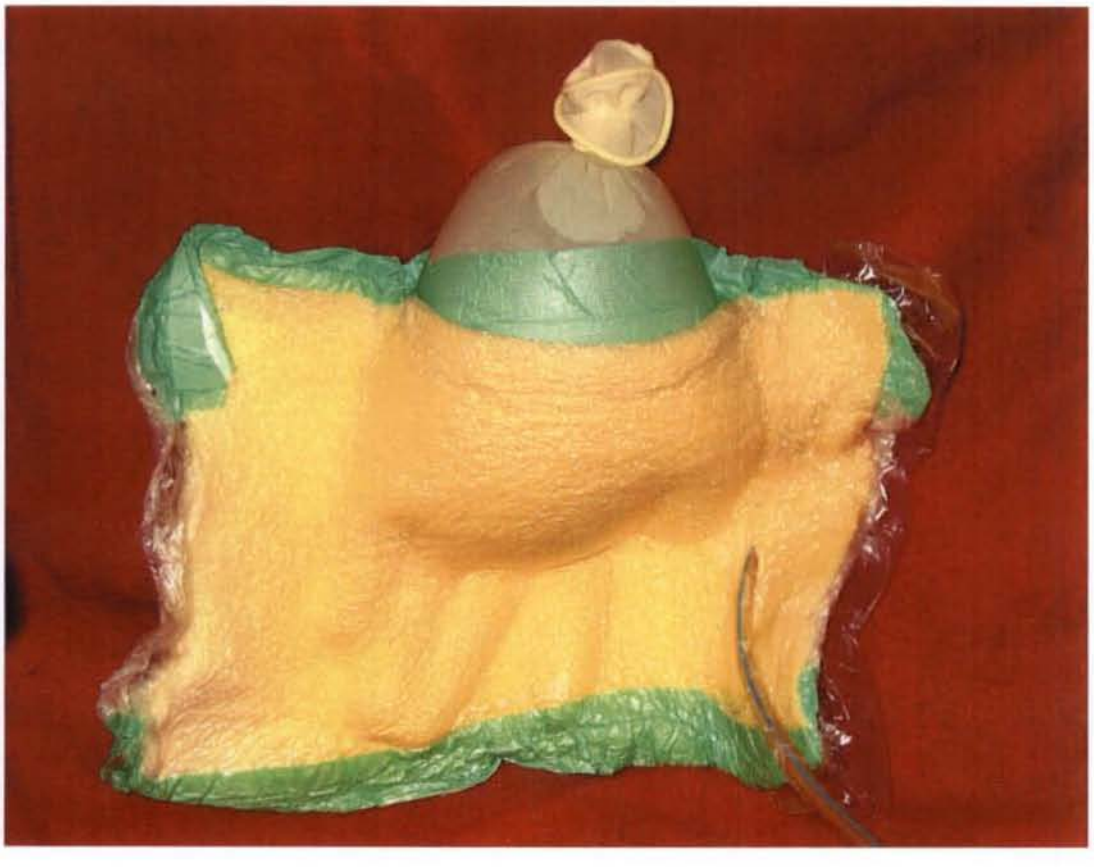

Fig. 31: Sandwich NPWT dressing applied to glove filled with water, with -125 mmHg of suction applied. Notice that there is no noticeable displacement of fluid from between the slabs of foam.

It appeared from this experiment that the latter hypothesis was true, namely that the force is applied to the foam until it collapses fully and then no further. However, there was the possibility that there were, in fact, forces being applied to the foam, but so miniscule that these were not visually noticeable. It was therefore decided to use a portion of the glove which required far less force to displace fluid, namely the finger.

(B) NPWT to finger of glove

To confirm the above mentioned hypothesis, a sandwich-type circumferential NPWT dressing was applied to the finger of the glove. If the former theory was true (atmospheric pressure would apply an unabated force) then the finger would be flattened between the two slabs of foam. However, if the latter theory was true, then the foam would collapse (as completely as possible) onto the glove surface and the finger would retain its shape and calibre.

Prior to application of the NPWT dressing the finger diameter was measured to be 2 cm. A sandwich-type NPWT dressing was applied using generic foam (Fig. 32). On application of suction the calibre of the glove finger could be clearly seen through the foam. It retained its diameter of 2 cm (Fig. 33). This appeared to confirm the previous experiments findings that the latter theory was true, namely the force is applied to the foam until it collapses to its maximal extent and then no further. Interestingly, the glove finger was noticeably shortened due to the horizontal (contractile) forces of the foam.

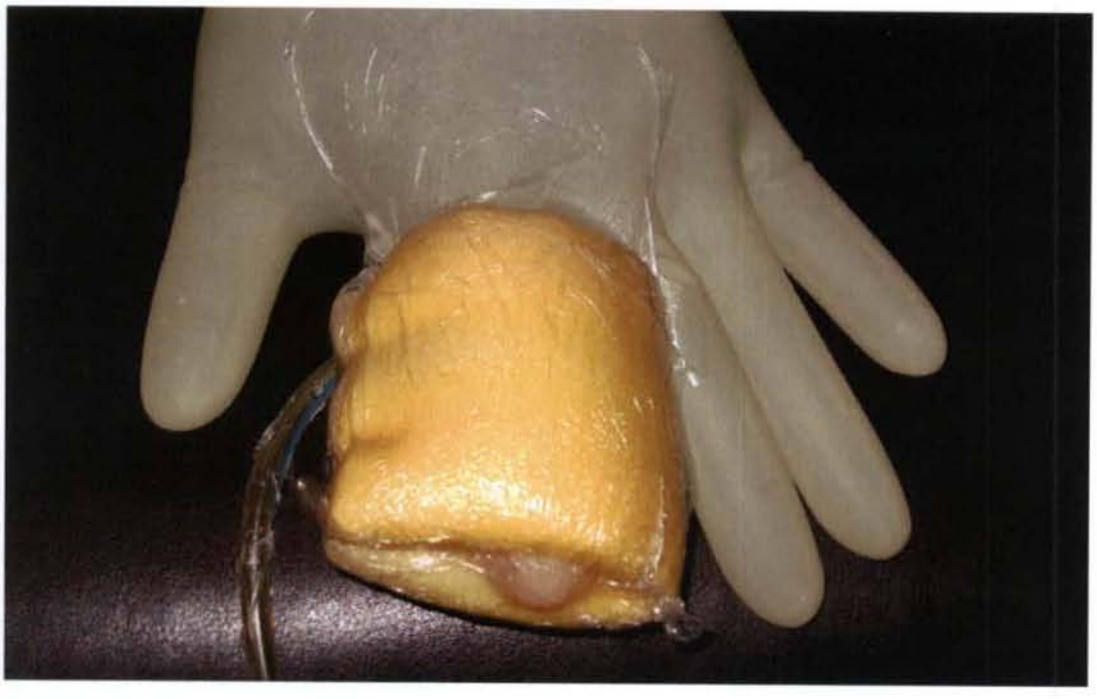

Fig. 32: Sandwich-type NPWT dressing (suction off) applied to the finger of a glove filled with water.

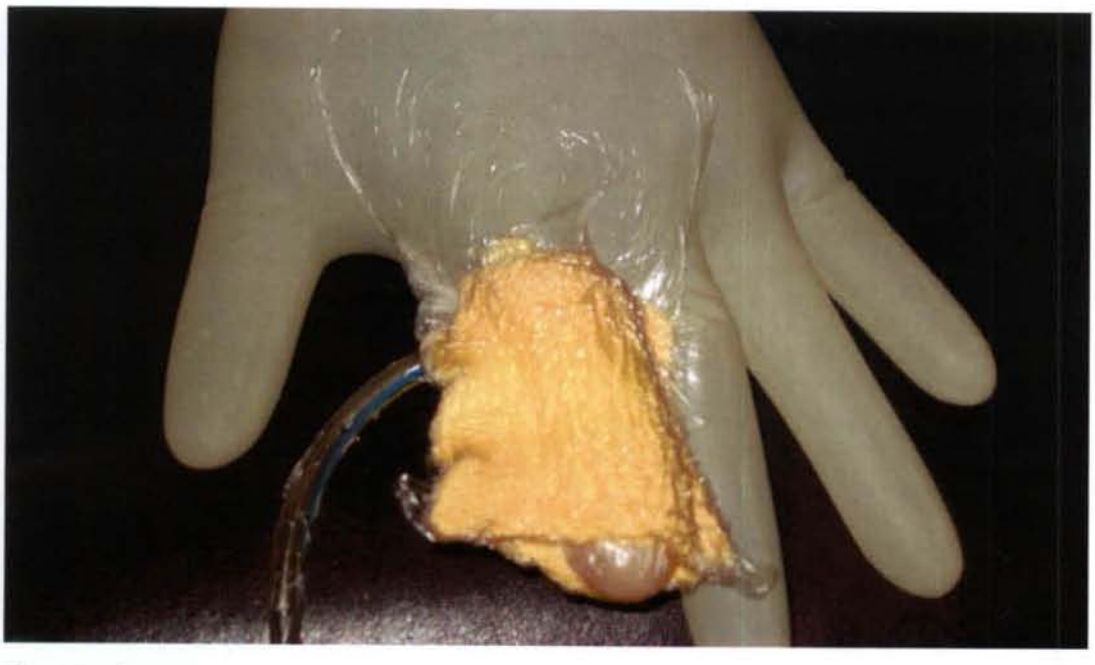

Fig. 33: Sandwich-type NPWT dressing applied to the finger of a glove filled with water with suction at -125 mmHg. Notice the finger diameter is identical to that of the other fingers. Also, notice the shortening of the finger due to foam contraction (horizontal forces).

The finding that the glove finger was not flattened was in keeping with our understanding of the laws of physics. The force applied to the foam is due to the pressure differential between dressing and atmosphere; however, there is also a pressure differential between the glove and the hypobaric dressing. The latter force vector is in the opposite direction to that of atmospheric pressure; hence there is no distortion of the glove.

The question is then raised as to why the Vacolitre experiments demonstrated increased pressure when the Vacolitre was exposed to a similar type of dressing. The likely answer is that the foam is probably displaced (albeit minimally) into the substance, creating an increase in substance pressure. This displacement was probably too small to be visible in the glove or Vacolitre experiments, although the pressure as a result thereof was, however, recorded to be increased in the latter.

3.3.12. Pressure effects of polyurethane foam vs generic foam

If it were to be assumed that the pressure generated within the substance undergoing NPWT was partly due to the interface dressing being displaced into the substance, then the net volume occupied by the substance (when totally collapsed) should play a role in how much pressure is generated within the substance , as it is this volume that is pushed into the substance. The commercially available reticulated, open-cell polyurethane foam (Kinetic Concepts, Inc., San Antonio, TX, USA) has pores that are approximately 400 – 600 μm in diameter.[1] Although the pore sizes of the generic foam used in this experiment are not known, they are visibly considerably smaller, implying that the foam material to pore ratio is greater. This implies that the generic foam with smaller pores has a greater material volume than that of the commercial foam, which in turn, implies that this ought to generate greater tissue pressure as it is pushed into the underlying tissues during NPWT.

An experiment was therefore conducted to compare the two in terms of the pressure they generate in underlying substances. Two identical experiments were conducted in parallel. Two identical cubes of processed meat (each measuring two cubic centimetres) were placed on the same rigid board. A microsensor (Codman/Johnson and Johnson Professional Inc., USA) was placed in each cube of meat and attached to two monitors for simultaneous monitoring. Two identically sized foams (5x10x3.3 cm), one generic and one reticulated, open-cell polyurethane foam (Kinetic Concepts, Inc., San Antonio, TX, USA), were placed over the cubes of meat and the NPWT dressing was completed as usual.

The suction tubes of the dressings were attached to a common suction tube via a Y-connector, so that suction could be applied simultaneously and with identical pressures. After the sensors were zeroed, suction was applied at -100 mmHg increments up to -500 mmHg, and the substance pressure was recorded.

On application of suction, the pressure in both cubes of processed meat increased, but less so in the one covered by generic foam (Fig. 34). As the suction pressure increased, the pressure increase in the meat covered by KCI foam appeared to be near-linear, whilst that in the generic foam appeared to start reaching a plateau at about -400 mmHg (Fig. 35).

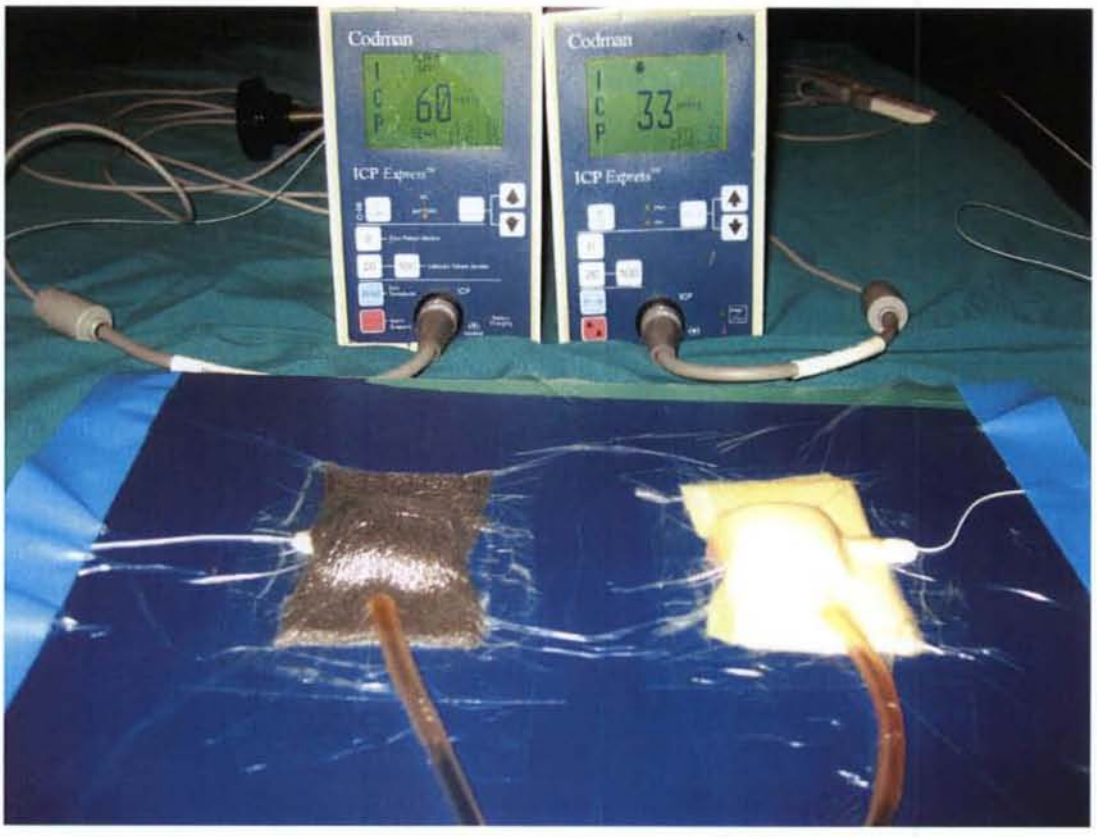

Fig. 34: Cubes of process meat covered by different foams (black reticulated, open-cell polyurethane foam (Kinetic Concepts, Inc., San Antonio, TX, USA) and generic foam) of identical size. Note the greater pressure increase in the meat covered by the polyurethane foam during application of equivalent suction pressures.

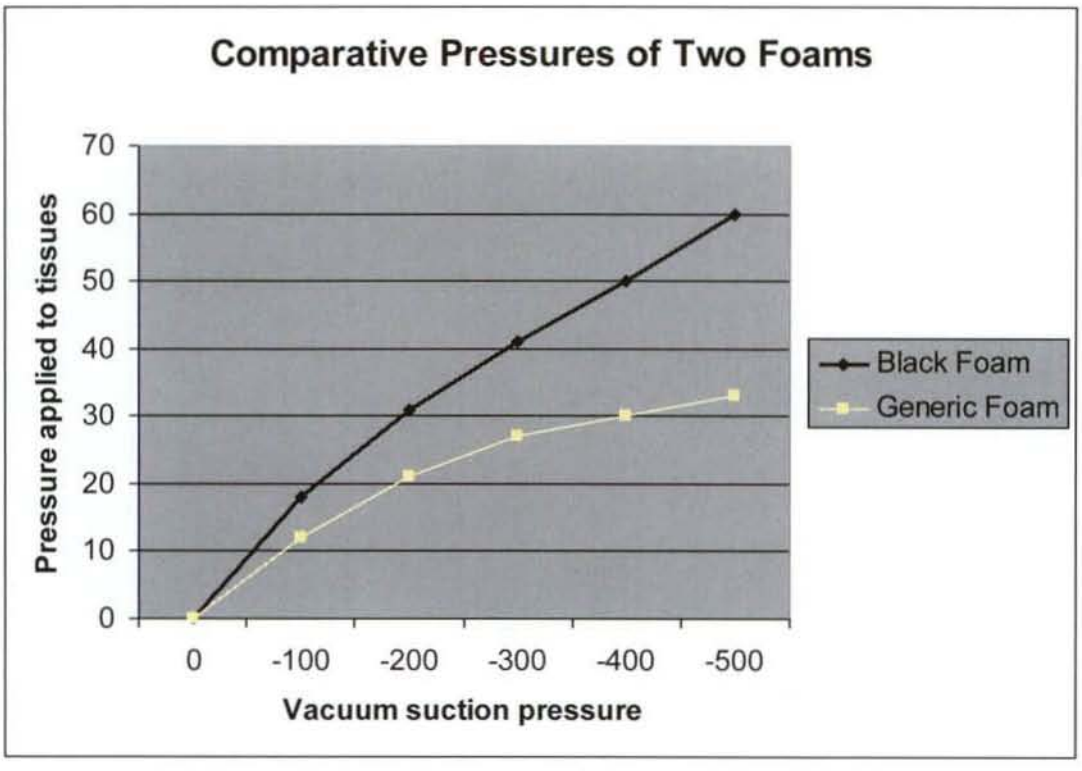

Fig. 35: Graph depicting the pressure increase in processed meat undergoing various levels of suction with two types of foam (black reticulated, open-cell polyurethane foam (Kinetic Concepts, Inc., San Antonio, TX, USA) and generic foam). Note the near-linear pressure increase in the processed meat covered by the polyurethane foam.

This experiment implied that the type of interface dressing influences the amount of pressure generated within the underlying substance. Although it was postulated that the foam that occupied the least volume when fully collapsed would result in the least substance pressure, this experiment appeared not to support this. Perhaps other factors, such as foam rigidity, open-cell technology, etc., are variables that play a greater role then foam volume and further research is required to clarify this.

3.4. Force studies

Although it appeared that NPWT generally tended to increase the underlying tissue pressure, the physics underlying this was still not clear. Whether this

was because of the proposed horizontal forces of the adhesive drape (during contraction of the interface dressing), which forced the surrounding tissues toward the dressing (thereby compressing them) or the vertical compressive forces of atmospheric pressure attempting to equalise the pressure differential on either side of the adhesive drape, was still not known. Indeed, it was likely that both forces acted simultaneously, to different degrees.

The pressure sensor used in the previous experiments was influenced by both vertical and horizontal forces, as both influenced tissue pressure. To evaluate the different types of forces in isolation, a force sensor was used, which was only receptive to unidirectional forces. The purpose of the sensor was to measure the vertical force applied by the interface dressing to the underlying substance during NPWT. As it would be placed between the interface dressing and the underlying substance, it was a requirement that the force sensor be as flat as possible (ideally flush with the surface) and thereby occupy as little space as possible between the two.

As previously mentioned, the space occupied by a sensor and the very presence of the sensor itself is likely to influence recordings of pressure or force, therefore the more flush the sensor was with the substance, the closer the model would be to a "real life" scenario where no sensor exists between the two. Piezo-electric force sensing resistors (Interlink Electronics, Camarillo, CA, USA) were used in the following experiments, as they are "paper-thin" (0.2 – 0.3 mm thick), flexible and allow for real-time measurement of force. Sensors were calibrated using known weights. Newton (N) was chosen as the unit of measurement.

3.4.1. Rigid surface experiments

The purpose of these experiments was to confirm that a downward force does, indeed, exist. Secondarily, in an attempt to gain insight into the physics behind the downward force and what influences this force, different variables were

assessed, namely suction pressures, foam types, foam volumes and dressing configurations. In the pressure experiments, many of these variables were also assessed but as the outcome measure was pressure, the directionality of the forces which caused the pressure changes could not be distinguished. The following experiments would be assessing only the downward (vertical) force of the NPWT dressing.

(A) Comparison of NPWT and manual pressure

Commercially available reticulated, open-cell polyurethane foam (Kinetic Concepts, Inc., San Antonio, TX, USA) was used to confirm whether a downward force exists. A table top was used as the rigid surface on which NPWT would be applied. A square of foam, measuring 4x4 cm was cut from a 3.3-cm thick slab. With the force sensor placed beneath the foam, the NPWT dressing was completed in the usual fashion (Fig. 36). There was no recordable increase in force prior to application of suction.

Fig. 36: NPWT dressing square with force sensor beneath it during application of suction.

A suction pressure was applied at -125 mmHg and a downward force was confirmed, measuring approximately 1.1 N. After the suction was disconnected the force immediately reduced to zero again. It was possible to reproduce this recording by applying manual downward pressure to the dressing. The aforementioned sequence was then repeated and it was noted that similar forces were recorded (Fig. 37).

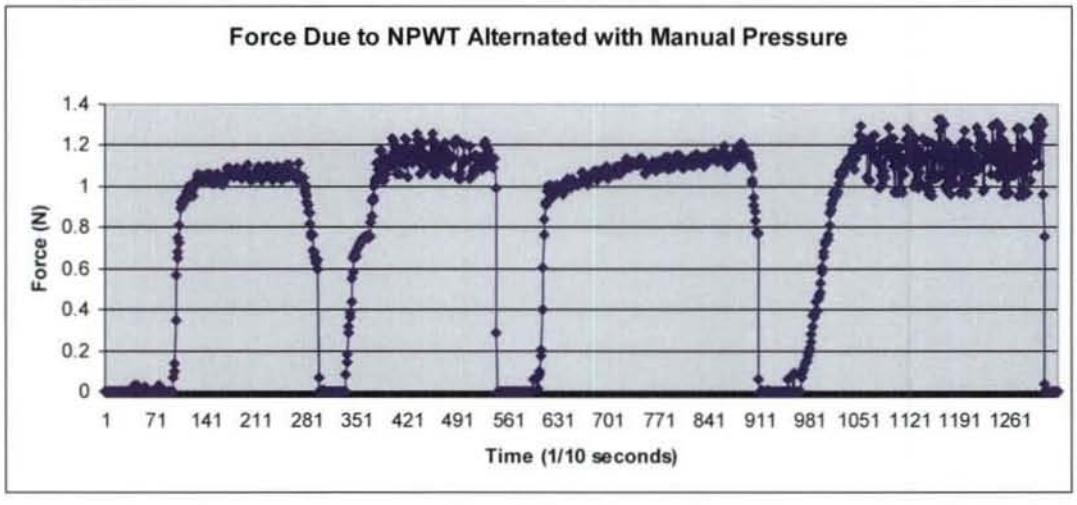

Fig. 37: Graph of force increments recorded during repeated sequence of suction at -125 mmHg, followed by short interval of no suction, followed by manual pressure to reproduce same force (two repetitions of this sequence).

This demonstrated that NPWT did, indeed, create vertical forces and that these are no different to manual downward pressure.

(B) Square of polyurethane (PU) foam and polyvinyl alcohol (PVA) foam

An experiment was carried out to compare the two most commonly used commercially available foams, namely PU foam and PVA foam. Each square of foam was 4x4 cm, but the PU foam was 3.3 cm thick, while the PVA foam was 1.2 cm thick. A suction pressure of -500 mmHg was created to allow the each foam to collapse as much as possible and record the force this could create. The PU foam generated vertical forces in excess of 3 N (Fig. 38), while the PVA foam

created less than 0.8 N of force (Fig. 39).

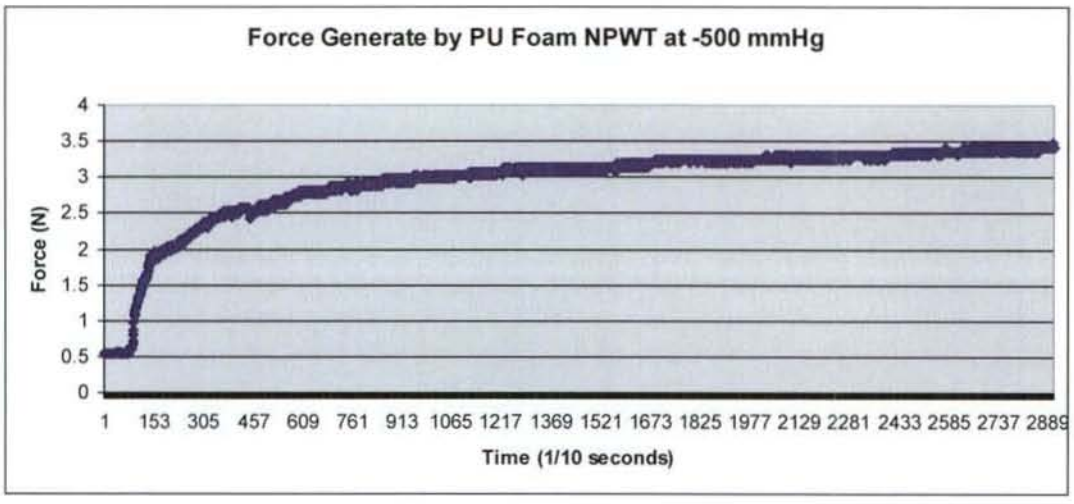

Fig. 38: Graph depicting the force generated by 4x4 cm square of PU foam at a suction pressure of -500 mmHg.

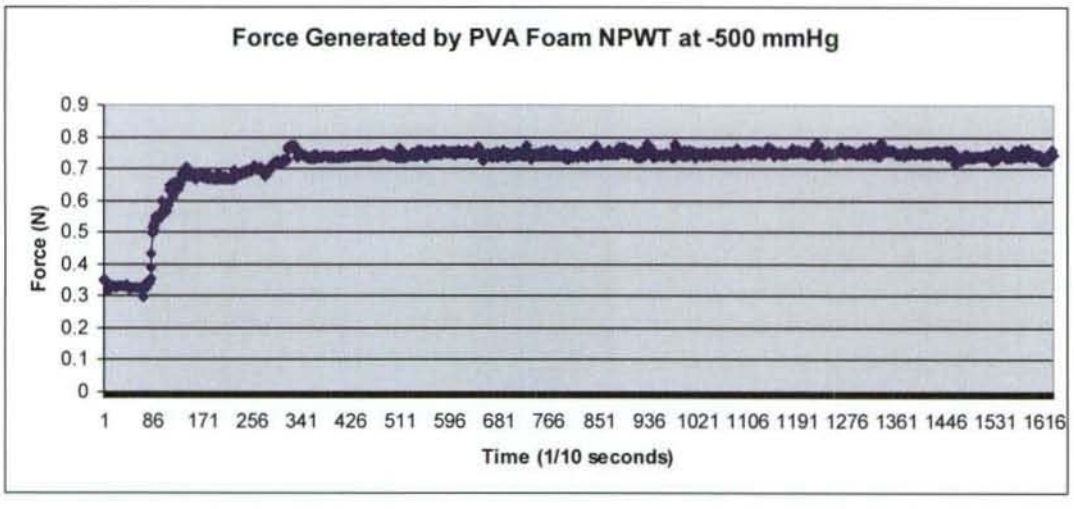

Fig. 39: Graph depicting the force generated by 4x4 cm square of PVA foam at a suction pressure of -500 mmHg.

These experiments demonstrated that the two most commonly used NPWT dressings would generate different amounts of vertical force at the same suction pressure. However, whether this was due to the different degrees of thickness (resulting in different foam volumes) or the type of material or both could not be

ascertained from this.

(C) Comparison of forces generated by identical squares of PVA foam on flat, convex and concave surfaces

The previous experiments confirmed that a downward (vertical) force exists following the application of suction to a NPWT dressing. The question arose whether this force would be identical if the surface underlying the dressing were not flat. It was postulated that a convex surface is likely to result in more force being generated than that seen on a flat surface during the contraction of the foam. A concave surface, on the other hand, may result in the opposite, because the foam may tend to "bowstring" away from the concavity as it contracts, resulting in less force being generated beneath it than that seen on a convex surface.

A 5x5 cm square of PVA foam (1.2 cm thick) was used as an interface dressing in all experiments and the NPWT dressing was completed as usual, with the force sensor beneath the dressing in the centre of the foam. A table was used for a flat surface, the outside of a plastic pipe (5 cm diameter) for a convex surface, and the acutely curved inner aspect of a dessert bowl for the concave surface.

Suction pressures were gradually increased through a range from 0 mmHg to -500 mmHg at -100 mmHg increments. Each suction pressure was held for one minute before moving to the next. Once the maximum suction pressure was reached, the reverse was carried out by decreasing the pressure in 100 mmHg increments with one minute in between. The latter component of the experiment was to assess whether the sensor consistently recorded a specific force for a specific suction pressure.

On the flat surface, the pyramid-like graph illustrated an increase in force as the suction pressure increased (Fig. 40). The maximum force was approximately 1.6N at -500 mmHg. The changes in force during the incremental changes in

suction pressure could be seen graphically as steps. It was noteworthy that the greatest changes in force were in the lower suction pressure ranges. In the higher suction pressure ranges the foam is likely to have already maximally collapsed, offering little additional force generation when more suction is applied.

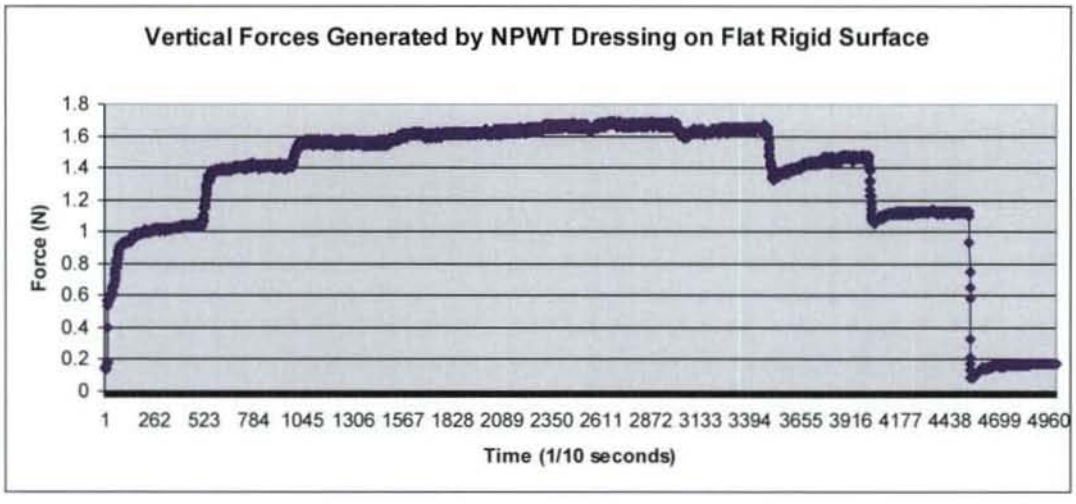

Fig. 40: Graph depicting the vertical forces applied to a flat surface by a NPWT dressing during an increasing range of suction pressures, followed by a decreasing range of suction pressures.

On the convex surface, a similar pyramid-like force graph was seen; however, the maximal force reached was only approximately 1.15 N, contrary to expectations (Fig. 41).

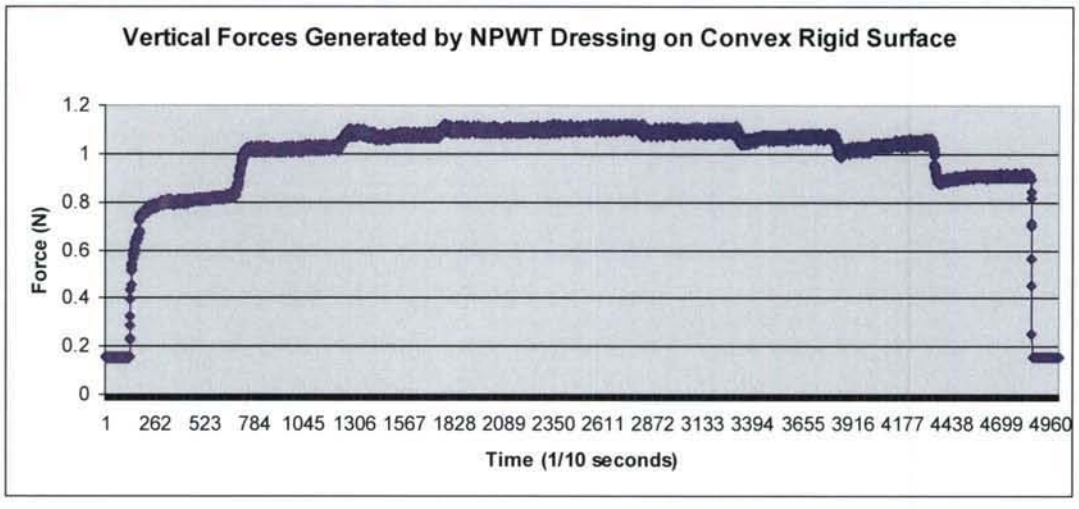

Fig. 41: Graph depicting the vertical forces applied to a convex surface by a NPWT dressing during an increasing range of suction pressures, followed by a decreasing range of suction pressures.

The vertical forces applied to the concave surface also resulted in a pyramid-like graph (Fig. 42), with the maximal force being approximately 3.3 N. Contrary to expectations, this experiment resulted in the greatest force increase.

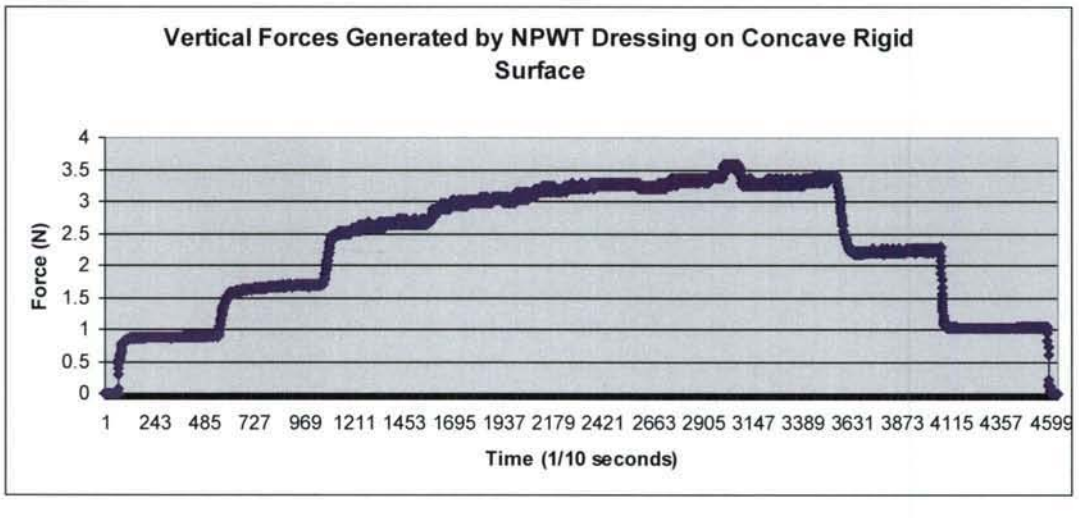

Fig. 42: Graph depicting the vertical forces applied to a concave surface by a NPWT dressing during an increasing range of suction pressures, followed by a decreasing range of suction pressures.

These findings appear to disprove the hypotheses that greater forces will be generated on a convex surface and vice versa on a concave surface. It appears that the opposite tends to occur. The reasons for the findings in these experiments were not clear. It was also noted that, in all the graphs, the forces for a given suction pressure were always higher in the reduction slope than those in the increment slope. The fact that this occurred consistently in all experiments implied that this was not due to sensor irregularities. For this, too, there was no explanation.

In order to check the accuracy and consistency of the sensor, the experiment on the concave surface was repeated with another identical dressing. A specific suction pressure was targeted to see if this would result in the same force that was seen in the previous experiment. A suction pressure of -200 mmHg was chosen, which in the previous experiment resulted in an increase in force of approximately 1.6 N during the suction pressure incremental phase. The repeat experiment appeared to confirm this amount of force on the concave surface, at this suction pressure (Fig. 43).

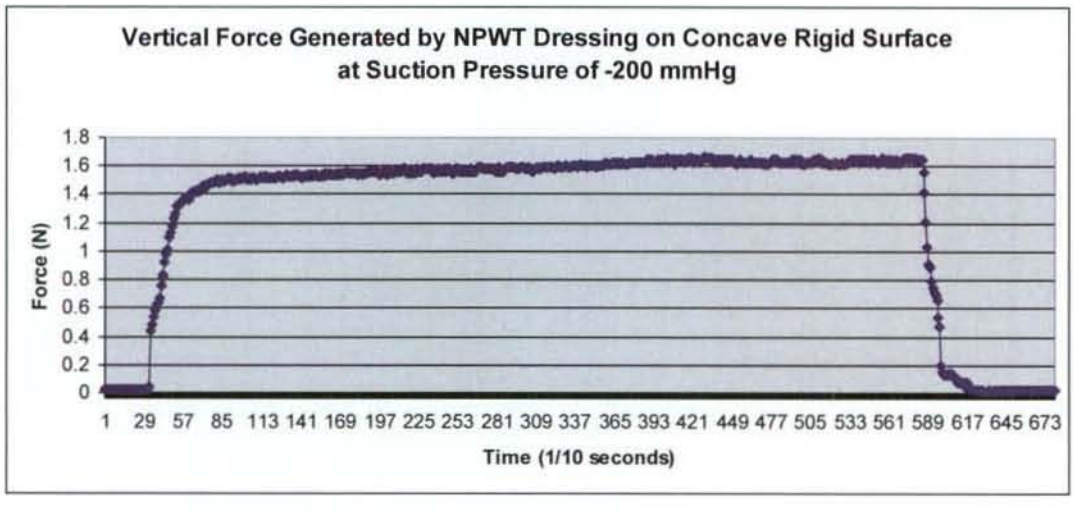

Fig. 43: Graph of the vertical force applied to a concave surface in a second NPWT dressing to validate the amount of force seen in the previous experiment, at a suction pressure of -200 mmHg. The amount of force appears similar for the given suction pressure, attesting to the reliability of the sensor.

It was also noted that despite the force reaching a plateau for a given suction pressure, the plateau in all the graphs tended to demonstrate a gently increasing slope. To what extent this would continue to increase was not known. Therefore another experiment was carried out on the flat rigid surface, using a NPWT dressing of the same dimensions. A suction pressure of -100 mmHg was applied and force was measured continuously for one hour. It appeared that the force gradually increased for the first eight minutes, after which it appeared to change very little (Fig. 44).

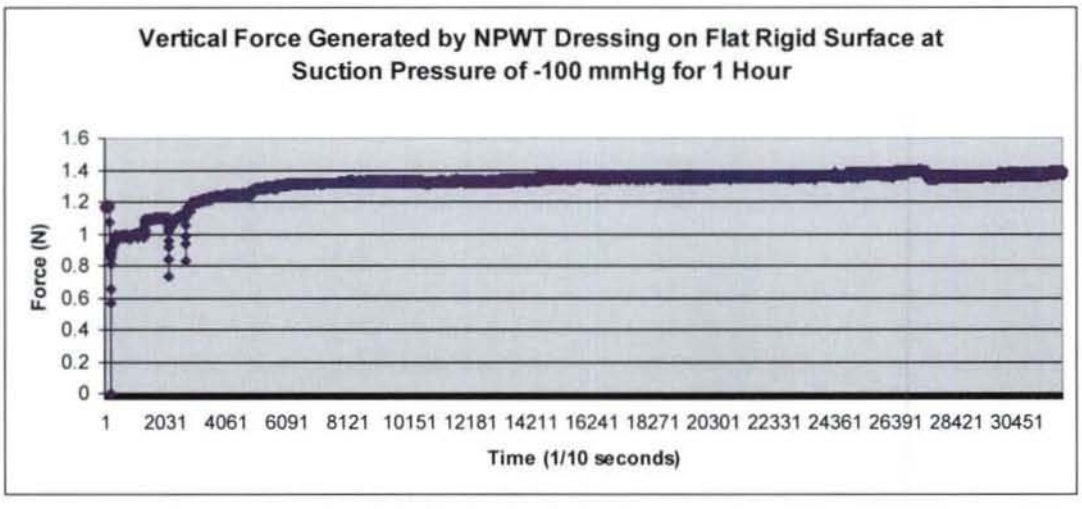

Fig. 44: Force generated by NPWT dressing on flat surface over one hour period at suction pressure of -100 mmHg.

3.4.2. Soft-surface experiments – pork meat

The previous experiments demonstrated the existence of a vertical force when suction is applied to a NPWT dressing. The rigid surfaces, however, eliminated the horizontal displacement seen in softer tissues during foam contraction. Whether this horizontal displacement, which is seen in softer substances, would reduce the tension in the adhesive occlusive drape, thereby reducing the vertical force applied, was not known. Furthermore, the amount of force registered by the sensor on softer substances may also be different to that seen on rigid surfaces

because the substance is more compliant. This will allow for a certain amount of "give" in the substance below the sensor, which may reduce the force recorded.

Experiments were therefore conducted using the same force sensor to evaluate the amount of vertical force recorded during NPWT on soft substances, in this case a leg of pork. The leg of pork used had a fairly flat surface (similar to that of the table experiments) on which to conduct experiments.

A two-part experiment was conducted. In the first component (A), a 2x2 cm "wound" was created through the skin (Fig. 45) and a similarly sized square of PU foam (3.3 cm thick) was placed over the force sensor in this wound to create a NPWT dressing. A suction pressure of -500 mmHg was applied and force was measured.

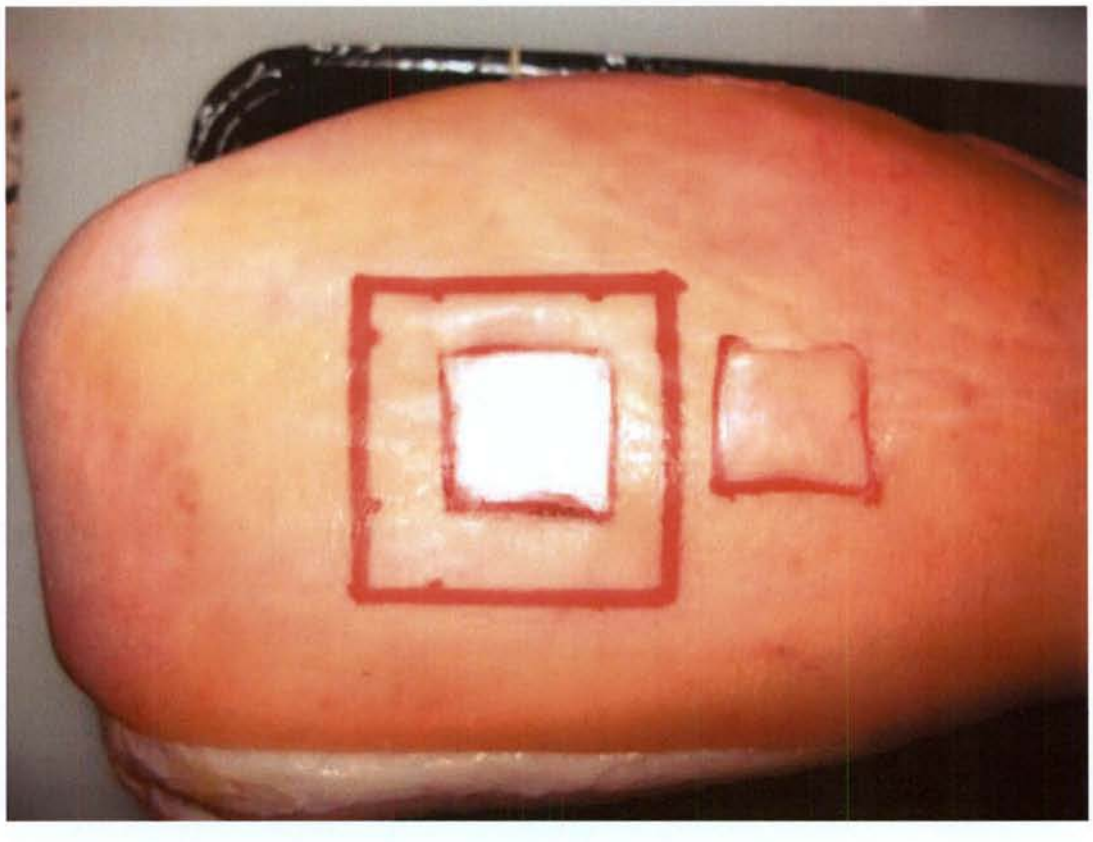

Fig. 45: A 2x2 cm area of skin removed from leg of pork in experiment (A) with the markings of a rim of a further 2 cm to be removed during the following experiment (B).

In the second part (B), the "wound" was doubled in size by excising a further 2 cm rim of skin and placing a 4x4 cm square of PU foam (3.3 cm thick) within this. The experiment was then completed as per the previous one (Fig. 46).

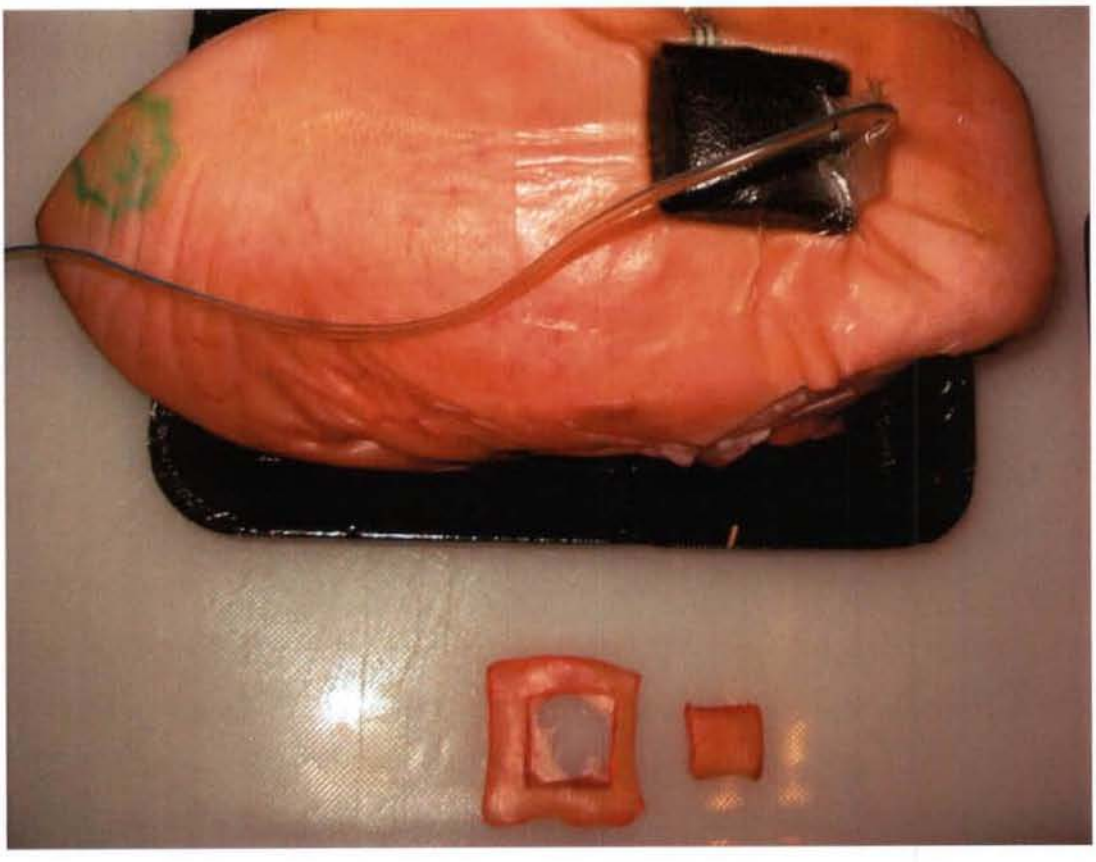

Fig. 46: A 4x4 cm area of skin removed from leg of pork in experiment (B) with NPWT dressing in place.

Prior to application of suction the sensor beneath the smaller foam (A) was already recording a force of 0.05 N and that of foam (B) 0.7 N. On application of suction the force generated by the smaller foam (A) increased to approximately 1 N, while the larger one (B) increased to approximately 3 N. This demonstrated that, as in rigid surfaces, a vertical force is generated in softer substances as well and that this appeared to be similar in magnitude to the force that was generated by a NPWT dressing of identical proportions on a rigid surface. Also

demonstrated was the fact that a larger volume of foam results in a larger amount of vertical force being applied to the tissues. Double the volume of foam did not result in double the amount of force, however. Although the smaller foam had a lower baseline force, it had a greater increase (20-fold) than that of the larger foam (4-fold).

The implications of these experiments was that, at a given suction pressure, the forces created on underlying tissues are not necessarily the same, as variables such as the curvature of the underlying surface, foam size and foam type, appear to influence this. This questions the universally accepted use of a suction pressure of -125 mmHg for all wounds, as recommended by Morykwas *et al.*[2] and Argenta *et al.*[1] In different NPWT dressing scenarios, it would be expected that the different forces being applied to wounds (despite the use of the same suction pressure), would affect wound healing to different degrees. Furthermore, this implied that if NPWT was applied to tissues with compromised perfusion, the size and type of foam used could influence whether capillaries would be occluded, due to the vertical forces applied to them.

3.5. Perfusion studies

The experiments done thus far suggested that NPWT does the opposite of what is commonly assumed. In other words, the application of suction to tissues via a NPWT dressing appears to increase tissue pressure, rather than reduce it. This conflicted with the fact that NPWT immediately increased perfusion on application of suction.[2-18]

3.5.1. Waterflow experiment

The fact that NPWT applied a vertical force to the tissues implied that the application of suction should reduce, rather than increase, perfusion. To test this, a laboratory experiment was undertaken to see whether NPWT had the capability

of compressing a rubber tube (representing a blood vessel), through which fluid was being passed

An elastic rubber tube, used by the physiotherapy department to exercise limbs, was attached to a rigid plastic board with Plasticine. One end of the tubing was attached to a giving set, which was attached to an intravenous infusion bag. The fluid in the bag was coloured with red food colourant to allow for easier visualisation during flow. A NPWT dressing using generic foam was placed over one portion of the rubber tube so that the tube would enter one end of the dressing and exit the other end.

The other end of the tube allowed for the fluid exiting the rubber tubing to pour into a tray. With the dressing in place, without suction, the fluid could be seen pouring into the tray at a constant distance (5 cm) from the edge of the plastic board (Fig. 47). This was due to the constant flow through the tube.

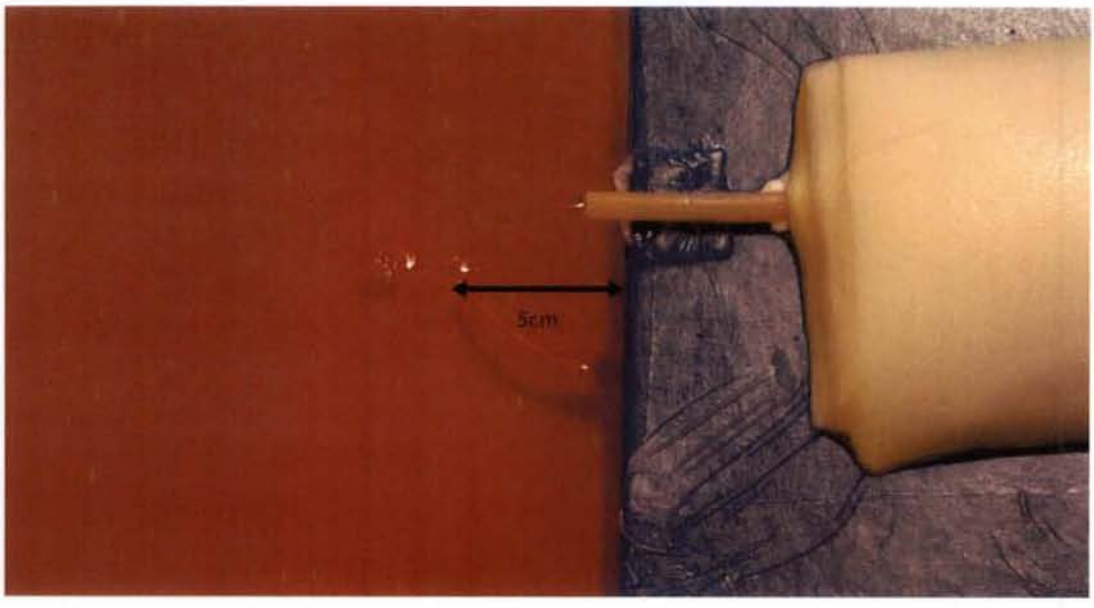

Fig. 47: NPWT dressing (without suction) applied over rubber tubing, through which coloured fluid is flowing at a constant rate, as evidenced by the constant pouring distance (5 cm) from the edge of the plastic board.

On application of suction at -125 mmHg, the pouring distance was less (3 cm), indicating that the flow rate through the tube beneath the dressing was reduced (Fig. 48). This was the first indication that NPWT may reduce perfusion by compressing blood vessels. However, the fact that this was done on a rigid surface raised questions regarding whether this could be extrapolated to living tissues.

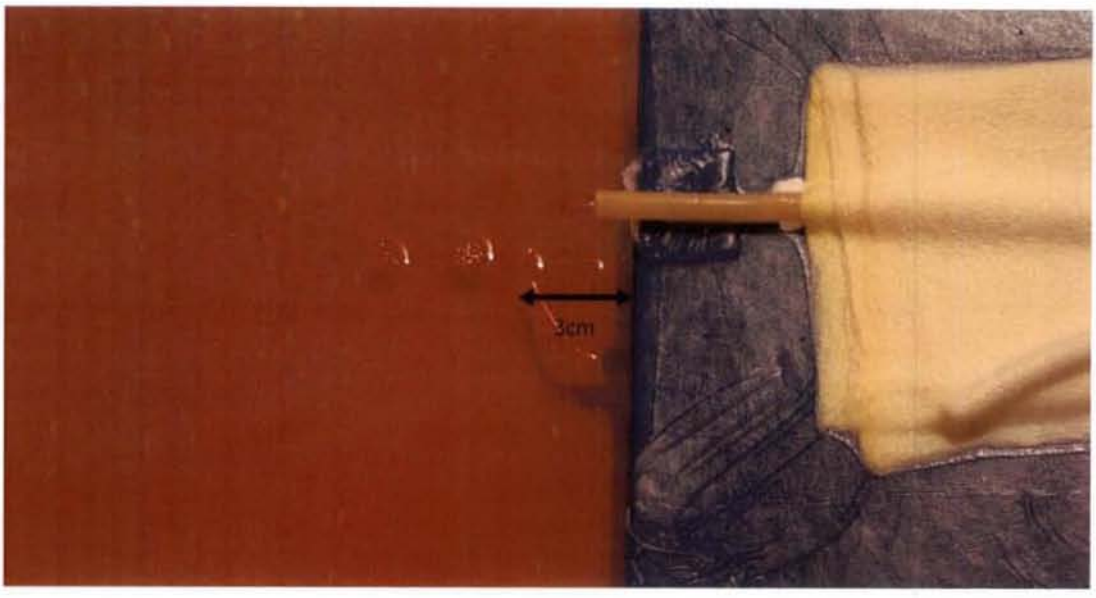

Fig. 48: NPWT dressing (with suction at -125 mmHg) applied over rubber tubing, through which coloured fluid is flowing at a constant rate. Note the reduced pouring distance (3 cm), compared to when no suction is applied (5 cm), indicating that the application of suction resulted in reduced flow through the tube.

3.6. Summary

Despite the informal nature of these experiments, a considerable number of interesting observations were made. In general, it appeared that NPWT tends to increase the pressure within the substance to which it is applied, unless the volume of the substance can be reduced by the NPWT dressing, by extracting fluid from the substance. This finding of increased tissue pressure was true for circumferential and non-circumferential NPWT. The increased pressures observed appeared to be the result of both vertical and horizontal forces on the underlying substance.

Factors that affect the pressure generated within the tissue appeared to be related to the:

- amount of suction utilised (greater suction resulted in higher tissue pressure)
- type of interface dressing (commercially available PU foam resulted in

higher tissue pressure than generic foam)

- volume of interface dressing (greater volume resulted in higher tissue pressure)
- type of substance undergoing NPWT
- skin laxity (the less adherent the skin was to underlying tissues, the less pressure generated during NPWT)
- continuity of skin around the tissues (absence of continuity may result in reduced tissue pressure increments during NPWT)

The forces created by NPWT dressings appeared to be able to reduce flow through a vessel carrying fluid.

However, due to the informal nature of these experiments, the scientific evidence to support the above-mentioned findings was not sufficient. Considering that the findings were in contradistinction to what was commonly assumed, the pressing need for formal research studies of a similar nature was realised. This led to the series of appropriately structured basic research experiments to be presented in the ensuing chapters.

3.7. References

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4 *Negative-pressure Wound Therapy and Tissue Pressure* – In vitro *Experiments*

Outline

4.1. Introduction

A common perception regarding the physics of negative-pressure wound therapy (NPWT) is that on application of suction, an area of hypobaric pressure is generated within the tissues in contact with the dressing, resulting in a pressure gradient being created with surrounding tissues.[1-7] This results in blood surging toward the wound and increasing perfusion. Reports published in the German literature, however, have found that tissue pressure becomes hyperbaric on application of NPWT.[8, 9] Preliminary, unpublished research at this unit, on inanimate substances exposed to NPWT, concurred with the German researchers.

The preliminary research undertaken at this unit, however, was often conducted on materials (Vacolitre intravenous infusion bags, oranges, bananas, etc.) that may have had limitations in their ability to transfer pressure homogenously within the material. In addition, the technique used to evaluate pressure was often primitive (manometry) or required the placement of a large sensor, which by virtue of its presence, may have affected the pressure being recorded. Therefore, prior to the initiation of this study, material was obtained that was considered appropriate for the abovementioned purposes. In addition, a sensor that occupied minimal space within the tissues and that could accurately measure hyperbaric as well as hypobaric pressure was also procured.

There are essentially three different configurations in which a NPWT dressing can be applied: foam placed circumferentially around the substance; foam placed non-circumferentially onto a substance; or foam placed in a cavity within the substance. It is quite conceivable that the pressure generated within the substance is entirely different for each of these different configurations. For example, foam placed around an object may increase tissue pressure on application of suction as a result of constriction, rather than due to suction itself. Similarly, foam placed inside a cavity would be expected to contract and reduce the tissue pressure of the surrounding substance. As these entirely different possibilities exist in each of

the three configurations, all three were tested in this study.

The objective of this study was therefore, to determine whether the application of increasing levels of suction to a NPWT dressing would reduce or increase the pressure within the substance to which it was applied; this was to be studied in circumferential, non-circumferential and cavity NPWT dressings.

4.2. Hypotheses

The following hypotheses were formed:

"The consequences of applying increasing amounts of suction to any NPWT dressing:

A: will always be to reduce the pressure of the substance to which it is applied

B: will either increase or decrease the substance pressure, depending on the configuration of the dressing (circumferential, non-circumferential or in a cavity)."

4.3. Methods

4.3.1. Instruments and materials

Substance pressures were measured using an intracranial tissue pressure microsensor (Codman/Johnson and Johnson Professional Inc., Raynham, Mass., USA), which makes use of a strain gauge transducer (Fig. 48). It measures both positive and negative pressures in gas, liquids or any compliant substances, such as soft tissue.

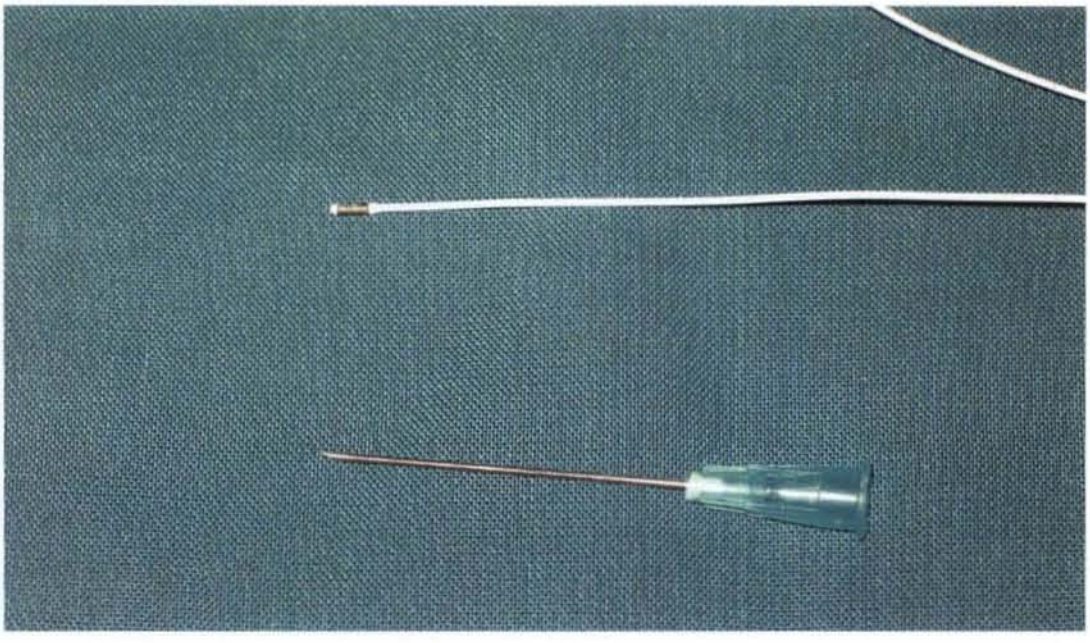

Fig. 49: Intracranial pressure microsensor with standard 21-gauge needle for size comparison.

The interface dressing used was generic foam, which was covered by an adhesive occlusive dressing (Opsite, Smith & Nephew, Hull, UK). A perforated suction tube was used to deliver the negative pressure to the dressing. Negative pressure was generated using a portable suction pump with an accurate pressure gauge (Schuco, Carle Place, N.Y., USA). In a pilot study it was found that conventional foam results in a similar change (albeit by different amounts) in substance pressure to that seen in commercially available reticulated, open-cell foam (Kinetic Concepts, Inc., San Antonio, TX, USA). All experiments were repeated five times and the means of these values were calculated.

4.3.2. Techniques

4.3.2.1. Circumferential NPWT

In order to simulate a limb, a large sausage was skewered onto a pen (which would represent the underlying bone). Using the supplied placement cannula, the pressure transducer was carefully placed in the substance of the sausage (Fig. 50).

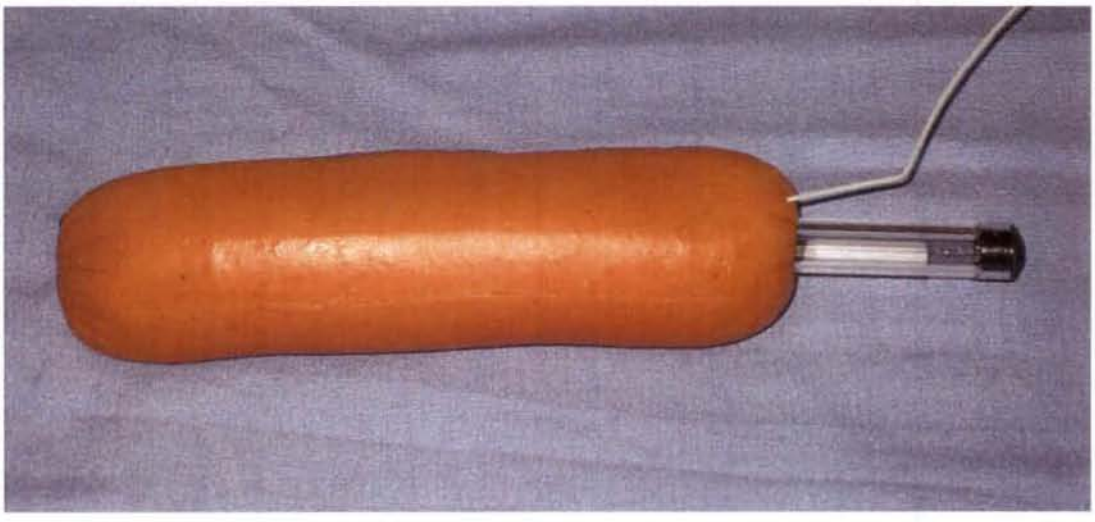

Fig. 50: Sausage skewered onto disposable pen to represent limb/finger with underlying bone. Pressure transducer inserted to a depth of about 5 cm into substance of sausage.

The transducer was placed about 5 cm from the puncture site (at one end of the sausage) and care was taken not to allow the transducer to be in continuity with the cavity created by the pen or the outer atmosphere. This would allow for true measurement of substance pressure alone. Rather than wrap the foam slab around the sausage, the sausage was loosely sandwiched between two separate slabs of foam. This was to create a circumferential NPWT dressing that would not constrict the sausage and thereby create a mechanical increase in substance pressure, which is unrelated to the changes due to the differential pressures. A portion of the sausage was left protruding from the foam and the adherent occlusive dressing was stuck directly onto this portion of the sausage, allowing part of the sausage to be excluded from the NPWT dressing, i.e. exposed to normal atmospheric pressure (Fig. 51), in the same way that a limb would not be entirely covered by a circumferential NPWT dressing. The transducer was zeroed in order to record the change in pressure that might occur. Different suction pressures were applied, ranging from -100 mmHg to -500 mmHg and the resultant substance pressures within the sausage were recorded.

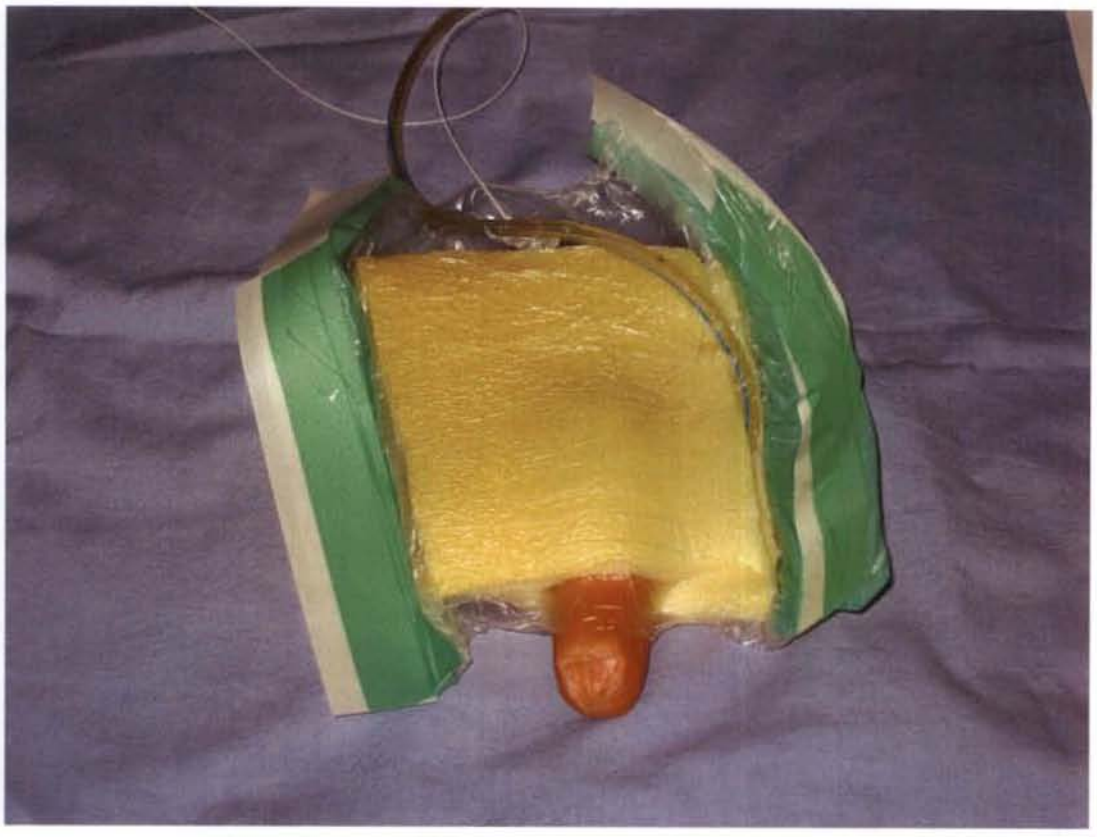

Fig. 51: Sausage within sandwich-type circumferential NPWT dressing. Portion of sausage is left in continuity with atmospheric pressure.

4.3.2.2. Non-circumferential NPWT

In order to determine whether non-circumferential NPWT dressings increase or decrease pressures within underlying substances, the transducer had to be placed within the substance of a compliant material. For this purpose, two slabs of soft Plasticine were used. The pressure transducer was placed between the two slabs, which were gently stuck together. A non-circumferential NPWT dressing was applied to the side of one slab (Fig. 52). If the NPWT dressing were to create a suction/pulling force on this slab, this would decrease the pressure on the transducer and vice versa if the dressing generated a pushing force, which would increase substance pressures. The transducer was zeroed and suction pressures ranging from -100 mmHg to -500 mmHg were applied to the dressing, with the resultant pressures within the Plasticine being recorded.

Fig. 52: Two slabs of soft Plasticine with pressure transducer in between and NPWT dressing over one slab.

4.3.2.3. NPWT in cavities

The material considered most suitable for this experiment was processed meat. As with the sausage experiment, this type of substance allows for the homogenous transfer of pressure. A round cavity (3-cm diameter, 2-cm depth) was excised from the meat with the rest of the outer plastic covering still intact. Because adhesive occlusive dressing does not stick to processed meat, this covering not only represented the surrounding skin of a wound, but also provided a surface for the adhesive occlusive dressing to stick to. From the opposite side of the processed meat, the pressure transducer was placed, with the help of the supplied placement cannula, about 1 cm deep to the base of the cavity. A cylindrical piece of foam was then inserted snugly into the cavity, with its outer surface flush with the surface of the processed meat. The rest of the NPWT dressing was completed with adhesive occlusive dressing and appropriately

sized suction tubing (Fig. 53). The transducer was zeroed and suction pressures ranging from -100 to -500 mmHg were applied, and the substance pressure in the base of the cavity was recorded.

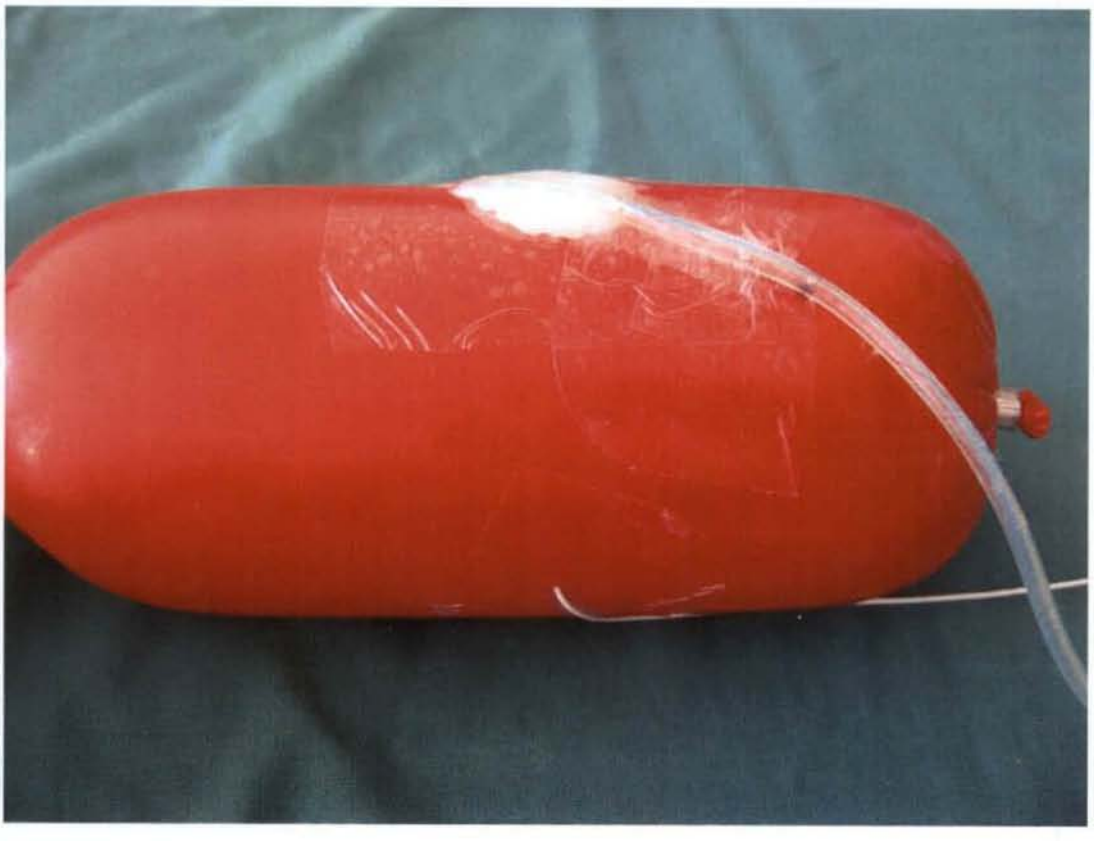

Fig. 53: Processed meat with NPWT dressing inside cavity. Pressure transducer introduced from opposite side and lodged about 1 cm deep to base of cavity.

This experiment raised the question of whether the pressures in the walls of the cavity were similar to the pressure in the base of the cavity, and whether this pressure dissipated as the distance from the cavity increased. To answer this question, a similar experiment to the aforementioned one was undertaken, except this time three transducers were used simultaneously and placed in the wall of the cavity, at a distance of 1 cm, 2 cm and 3 cm away from the cavity (Fig. 54). The transducers were zeroed and suction pressures ranging from -100 to -500 mmHg were applied, and the substance pressures at the respective distances

from the cavity were recorded.

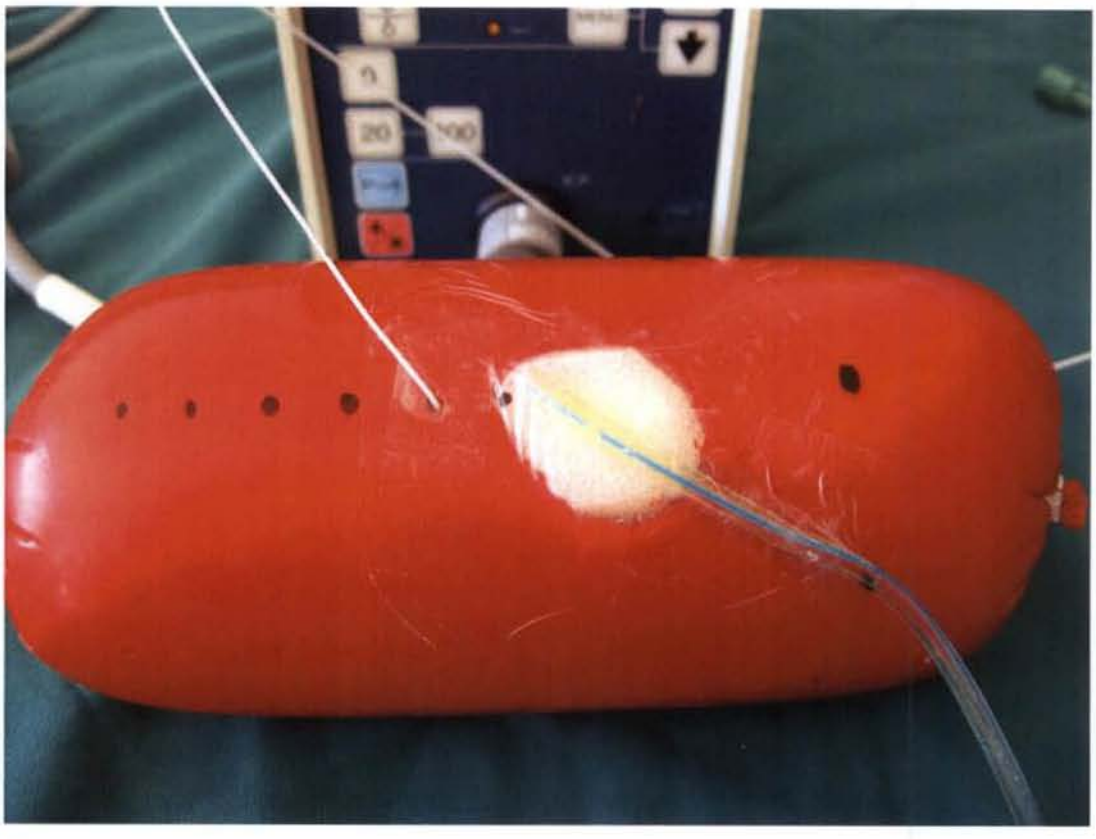

Fig. 54: Markings at 1-cm intervals away from cavity, at which point three pressure transducers are to be placed at a depth of 1 cm.

4.4. Results

4.4.1. Circumferential NPWT

Pressure inside the substance of the sausage increased proportionately with increasing suction pressure (Fig. 55). The high sensitivity of the transducer demonstrated that this increase occurred even at very low suction pressures, and at no time was a negative pressure recorded.

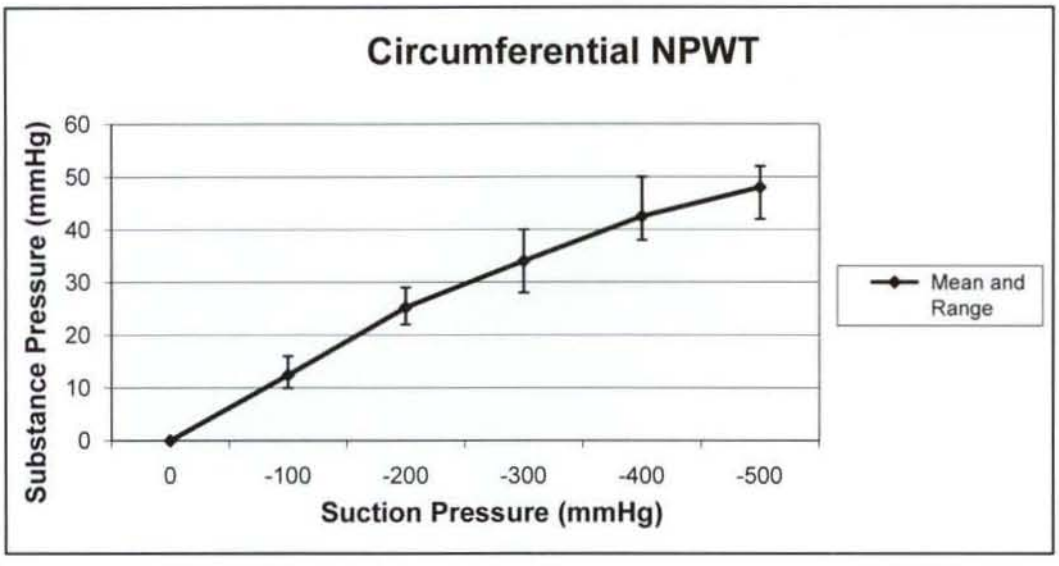

Fig. 55: Pressure change (mean and range when experiment repeated five times) in sausage substance in relation to increasing suction pressure (circumferential NPWT).

4.4.2. Non-circumferential NPWT

In the Plasticine, the transducer recorded a proportionate increase in pressure as the suction pressure was increased (Fig. 56). At no time was a negative pressure recorded.

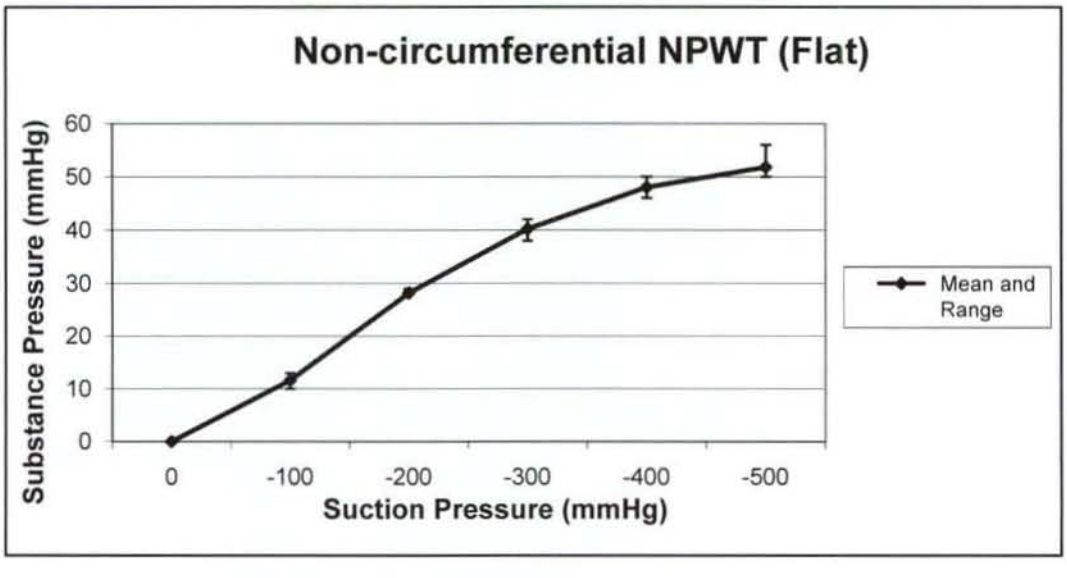

Fig. 56: Pressure change in Plasticine (mean and range when experiment repeated five times) in relation to increasing suction pressure (non-circumferential NPWT).

4.4.3. NPWT in cavities

The transducer in the base of the cavity recorded a proportional increase in pressure as the suction pressure was increased (Fig. 57).

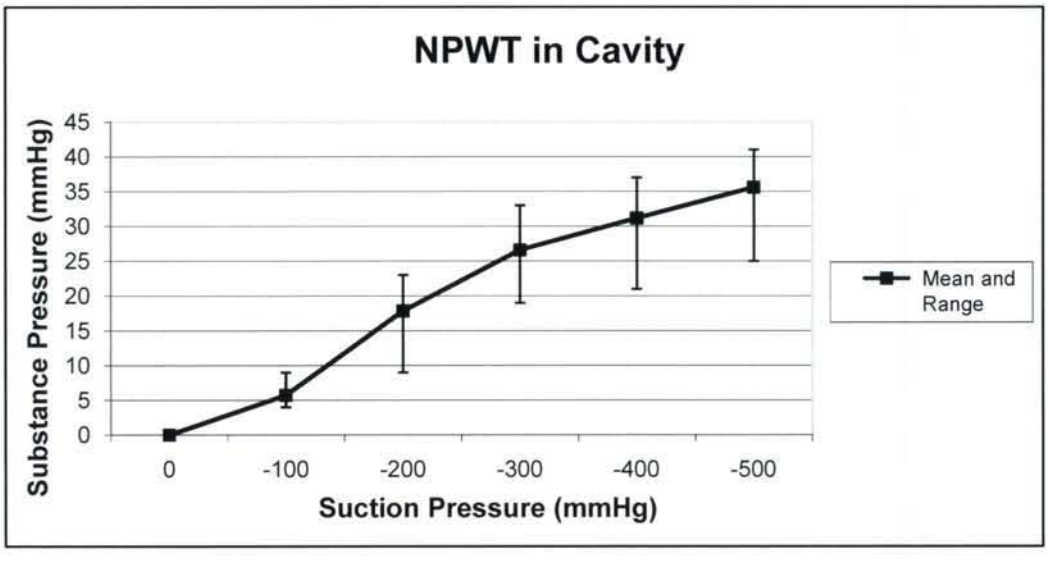

Fig. 57: Pressure change (mean and range when experiment repeated five times) in base of cavity of processed meat in relation to increasing suction pressure (NPWT foam placed in cavity).

The pressure recorded 1 cm from the wall of the cavity demonstrated an increase in pressure as well, although the rise in pressure was not as acute as in the base (Fig. 58). At both the 2-cm and 3-cm intervals the pressure rise was minimal and increasing suction did very little to generate higher pressures, indicating that pressure appears to dissipate very rapidly in this particular substance. There was no decrease in pressure, however, at any of the placement distances.

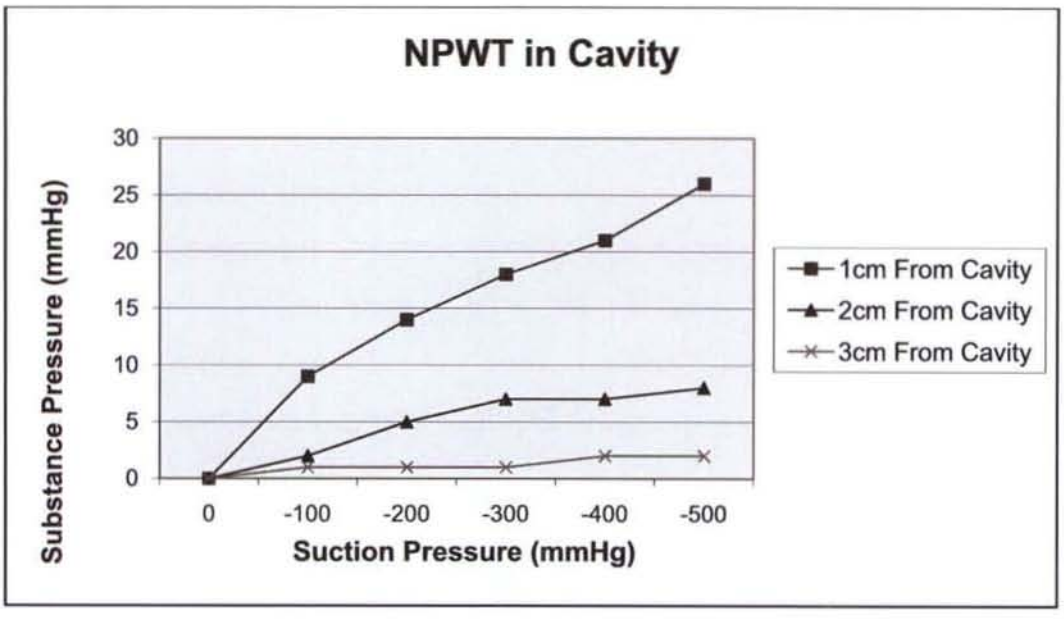

Fig. 58: Pressure changes in processed meat at varying distances from cavity, in relation to suction pressure (NPWT in cavity).

4.5. Discussion

The results of this study demonstrate that all types of NPWT dressings generate an increase in pressure within the underlying substance, regardless of what the particular substance is. The increase in pressure is directly proportional to the amount of suction pressure used. The increase in substance pressure dissipates as the distance from the NPWT dressing is increased.

These findings, however, conflict with other studies suggesting that NPWT creates a hypobaric tissue pressure, resulting in blood flow surging toward the wound.[1-7] As substance pressures have been demonstrated to be hyperbaric, the cause for increased perfusion (if present) seems unlikely to be directly due to NPWT. The sequelae of this increased tissue pressure may, however, indirectly result in increased perfusion at a later stage.

Hyperbaric tissue pressure and the potential for ischaemia, with release of vasodilatory mediators,[10] may explain the hyperaemia that is observed when the foam is removed. This potential for ischaemia has already been demonstrated by others.[11-15] The tissue ischaemia may further act as a stimulus for the increased angiogenesis observed.[10, 16-18] Additionally, it can be postulated that the increased pressure dissipates oedema fluid away from the wound. Should this fluid be dissipated to the wound surface, the NPWT dressing is then in a position to remove it from the wound. The decrease in wound oedema, with associated decompression of the tissues, may be one mechanism accounting for the increased perfusion observed after the application of NPWT over a period of time.

The increased substance pressure generated by NPWT and the potential for ischaemia raises concern about the safety of NPWT when applied to tissues with compromised perfusion. This has clinical relevance when applied over an avulsed flap of skin or any other traumatised tissue with borderline perfusion, particularly when used circumferentially.

A limitation of this study is that all substances tested are inanimate. Unlike living tissues, inanimate substances do not have various fluid compartments and may also not have the same visco-elastic properties that human tissues have. Therefore, the specific pressures observed in this study are not necessarily indicative of pressures that may be generated in human tissue. However, although the specific pressures may be different, the trend observed, namely increasing substance pressure for increasing suction pressure, is likely to occur in living tissues as well.

4.6. Conclusion

NPWT generates hyperbaric pressures in inanimate substances, regardless of method of application (circumferential, non-circumferential or in cavities). This increase in pressure is directly proportional to the amount of suction applied. The increased pressure dissipates rapidly as distance from the wound edge is increased.

Hypothesis (A) is therefore rejected as NPWT does not reduce the pressure in the substance to which it is applied. Whether NPWT increases or decreases substance pressure is NOT dependant on the dressing configuration – all types of dressing configurations increased the substance pressure, albeit to different degrees. Therefore, hypothesis (B) is also rejected.

These findings suggest that it may be necessary to reconsider our current understanding of the physics relating to NPWT. Furthermore, the hyperbaric tissue pressure generated by NPWT may be cause for concern regarding the role of NPWT in tissue with borderline perfusion and further studies on living tissues are warranted.

4.7. References

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5 *Negative-pressure Wound Therapy and Tissue Pressure* – In vivo *Experiments*

Outline

5.1. Introduction

The study conducted on soft, inanimate substances (see Chapter 4) confirmed that all types of NPWT dressing configurations increase the pressure within the substance to which they are applied. Although this is in keeping with work published in the German literature,[1,2] this study was criticised, not only because it conflicted with the common perception of how NPWT influences tissue pressure,[3-7] but also because it was conducted on inanimate substances. It was felt that these are not representative of the environment to be found within living tissue. Although living tissue also comprises a "soft substance", it also contains intercellular and intravascular fluid compartments. Due to the inherent properties of fluid, the application of NPWT to living tissue may result in an entirely different change in tissue pressure to that seen in inanimate substances. Indeed, some have compared living tissue which is undergoing NPWT, to a sponge which contains fluid;[8] in this case, application of suction should conceivably tend to extract the fluid and reduce the pressure within the sponge.

There was a need to determine whether the pressure findings within inanimate substances were also applicable to living tissues. Understanding how the pressure within living tissues responds to NPWT is fundamental to understanding its mechanism of action. The aim of this study was to assess whether human tissue pressures increase or decrease in response to varying degrees of NPWT. Again, as with the experiment on inanimate substances, three different dressing configurations were studied: circumferential; non-circumferential; and NPWT in a cavity.

Furthermore, research was lacking as to whether there may be a sustained pressure increase or whether it would eventually reduce (or even become hypobaric) over a period of time. Therefore, tissue pressure changes over the first 48 hours were also evaluated.

5.2. Hypotheses

Following the studies on inanimate substances, three hypotheses were formulated prior to this study:

A: "The pressure within living tissues will increase on application of increasing amounts of suction to a NPWT dressing."

B: "All three types of NPWT dressing configurations will result in an increase in tissue pressure as suction pressure is increased."

C: "The increased tissue pressure will always reduce over a period of 48 hours, when NPWT is applied at a set suction pressure."

5.3. Methods

5.3.1. Instruments and materials

As with the previous study on inanimate materials, three fundamentally different NPWT configurations were assessed: circumferential, non-circumferential and foam placed in a cavity. "Circumferential NPWT" implies that the interface dressing is in contact with the entire circumference of the limb. The interface dressing used was reticulated, open-cell, polyurethane foam (Kinetic Concepts, Inc. (KCI), San Antonio, TX, USA). When testing tissue pressure changes over 48 hours, a KCI pump, set at -125 mmHg was used. This pump does not generate pressures higher than -200 mmHg, and therefore, in order to test the effects of suction pressures higher than -200 mmHg (up to -450 mmHg), a medical suction pump (Schuco, Carle Place, N.Y., USA), with an accurate pressure gauge, was used.

"Tissue pressure" refers to the mechanical pressure measured within the tissues, i.e. the interstitial hydrostatic pressure. It is this pressure which, if greater than capillary intravascular pressure, will result in capillary occlusion. This was measured using an intracranial tissue-pressure microsensor (Codman/Johnson

and Johnson Professional Inc., Raynham, Mass., USA). This strain gauge sensor can be placed into fluids or parenchyma[9, 10] and is accurate to within ±1 mmHg.[10,11] It can measure both hyperbaric and hypobaric pressures, with a functional range of -50 mmHg to +250 mmHg.[11] The cable is 0.7 mm in diameter, with the transducing tip measuring 1.2 mm (Fig. 59).[11, 12] This miniscule calibre allows it to be placed into brain parenchyma with minimal tissue trauma. It has been shown to be highly accurate in human studies.[13]

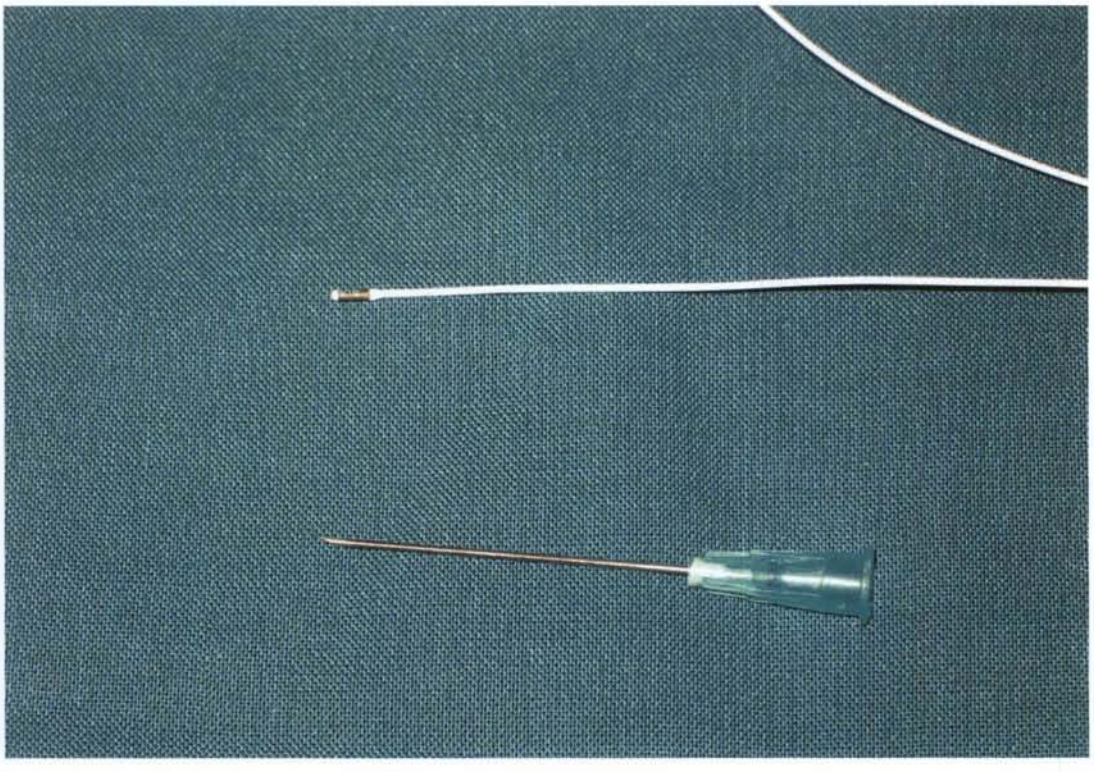

Fig. 59: Intracranial pressure microsensor with 21G needle for comparison

Measuring exact intracranial pressure is possible because the cranial vault is essentially a rigid container. In tissues, for example, a muscle, which are not encased in a rigid structure, pressure variations in response to an external force depend on where the measurement is taken. Therefore, for the purposes of this study, "specific pressures" were not considered as important as "pressure

changes" at a given point. For this reason the pressure transducer was zeroed after placement, allowing for recording of pressure changes above or below baseline pressures, rather than actual tissue pressures. To ensure correct functioning of the sensor *in situ*, the transducer was tested after placement by applying manual pressure to the tissues, which would result in increased tissue pressure measurements.

A total of 14 patients were recruited into the study, with one patient being used twice, with the sensor in different areas (15 tests). Four patients had wounds requiring a circumferential NPWT dressing, five had wounds requiring a non-circumferential NPWT dressing and the other five required a NPWT dressing to be inserted into a cavity. On every patient, the experiment involved two phases. The first phase was to determine how different suction pressures affect tissue pressure, while the second phase evaluated how tissue pressure changes over a period of 48 hours, at a set suction pressure.

5.3.2. Patients and techniques

5.3.2.1. Circumferential NPWT dressings

The wounds that required circumferential NPWT were all hand injuries, most of which had relatively contaminated wounds that precluded immediate reconstructive surgery (Fig. 60).

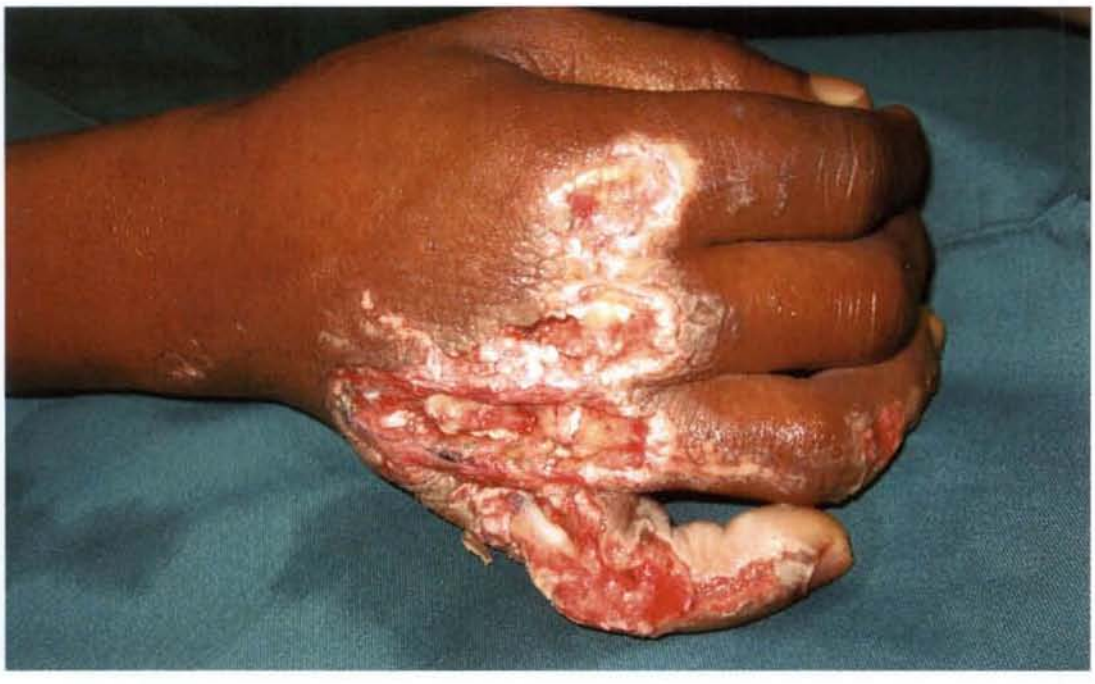

Fig. 60: Deep abrasion on hand dorsum. Application of a circumferential NPWT dressing is not only beneficial to the wound, but reduces oedema in the entire hand.

After initial debridement of the wounds, the microsensor was placed via the existing wound into the surrounding tissues, at different anatomical locations in each hand. Care was taken not to allow the sensor to be in communication with the air pressure over the wound, as this would be a measurement of the suction pressure rather than the tissue pressure. This was achieved by ensuring that the distance from the site where the sensor was introduced into the tissue to the position of the sensor within the tissue, was a minimum of 30 mm. This was considered ample distance, given that Murphy *et al.* found that the sensor did not record any negative-pressure beyond 1 mm from site of insertion.

The first case had undergone flap surgery a week earlier to resurface a wound but was complicated by partial necrosis of the flap. The necrotic portion was debrided and the sensor was placed beneath the remaining viable flap and a circumferential NPWT dressing (Granufoam Hand Dressing, Kinetic Concepts, Inc., San Antonio, TX, USA) was applied. The Granufoam Hand Dressing has the

advantage that the hand is sandwiched between two separate slabs of foam. If a single slab of foam was merely wrapped around the hand, the reduction in size of this interface dressing could confound results by constricting the hand. One week later this patient was taken to theatre again to cover the remaining palmar wound with a full thickness skin graft (FTSG). A circumferential NPWT was again used, this time to secure the graft. On this occasion the sensor was tunnelled and placed beneath the dorsal skin of the proximal phalanx (P1) of the index finger. The second patient had the sensor placed beneath the skin overlying the dorsum of the middle phalanx (P2) of the middle finger. The third case had the sensor placed over the third metacarpophalangeal joint (MCPJ) and fourth case had the sensor placed in the second web space.

5.3.2.1.1. Phase 1

With the dressing in place, the transducer was zeroed in order to start the first phase. Suction pressure was then gradually increased from zero through to -450 mmHg with -75 mmHg increments. Tissue pressure changes were recorded for each increment of suction pressure. Suction was then switched off and tubing disconnected, allowing the foam to re-expand. After one minute the sequence was repeated again, for a total of five times. The means of the five values for each increment were calculated.

5.3.2.1.2. Phase 2

Following phase 1, the NPWT was then connected to the quieter KCl pump for the second phase of the experiment. This would avoid the patient having to endure two nights with the noisy Schuco pump. The suction pressure was set at the pre-selected suction pressure of -125 mmHg. Tissue pressure readings were recorded every hour for 48 hours. On completion of the experiment the NPWT dressing was removed, the transducer was withdrawn from the tissues and wounds were managed on their own merits.

5.3.2.2. Non-circumferential NPWT dressings

Any wounds that required a NWPT dressing which was neither circumferential nor placed in a cavity were included into this arm of the study (Fig. 61). The following cases were enrolled in this part of the study: a forearm wound, a scalp abrasion, a heel wound, and two thigh degloving wounds.

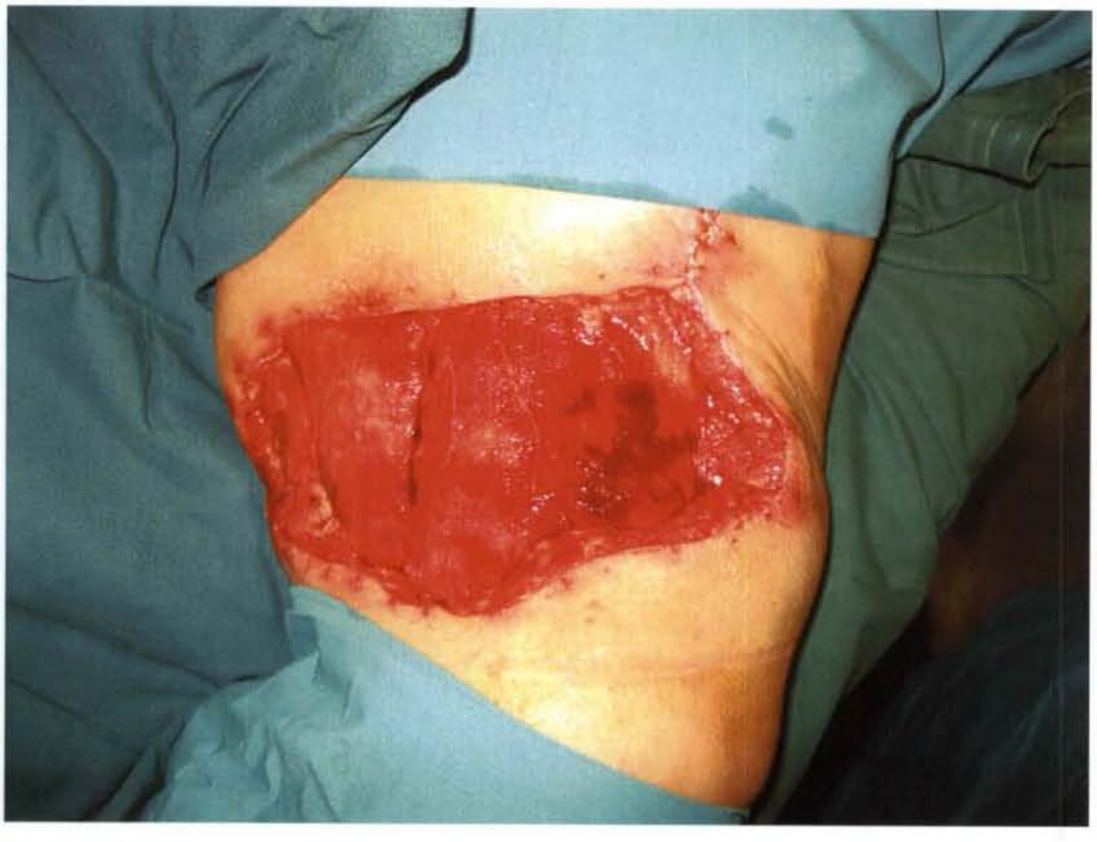

Fig. 61: Thigh wound on an obese lady prior to placement of a non-circumferential NPWT dressing.

Following intraoperative debridement, the transducer was inserted about 5-mm deep to the surface of the wound bed, with at least 30 mm between the sensor and the insertion site. The NPWT dressing was placed over the wound, following standard technique.

5.3.2.2.1. Phase 1

After the transducer was zeroed, Phase 1 followed the same sequence as Phase 1 in the circumferential NPWT group.

5.3.2.2.2. Phase 2

After the transducer was zeroed, Phase 2 followed the same sequence as Phase 2 in the circumferential NPWT group.

5.3.2.3. NPWT in cavities

The wounds that were selected for this part of the study were those that formed a deep cavity (Fig. 62). One ischial and three trochanteric bedsore cavities were included in this category. The fifth patient had a septic spinal wound dehiscence following excision of a meningioma.

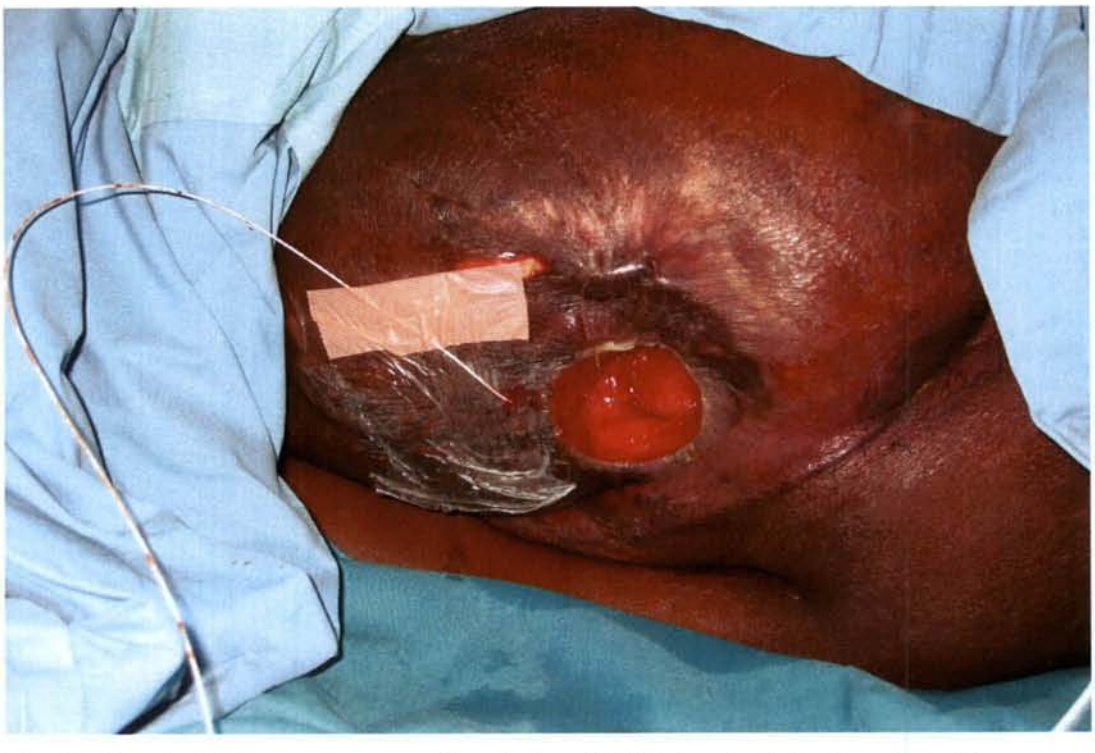

Fig. 62: Deep ischial pressure sore cavity prior to a NPWT dressing being inserted, with pressure sensor in place in the wall (1 cm deep to skin and 1 cm from cavity).

Unlike the previous two categories where most of the foam lies on top of the wound, these wounds result in the foam lying inside the wounds, with the outer surface of the foam being essentially flush with the skin prior to suction being applied (Fig. 63a and b).

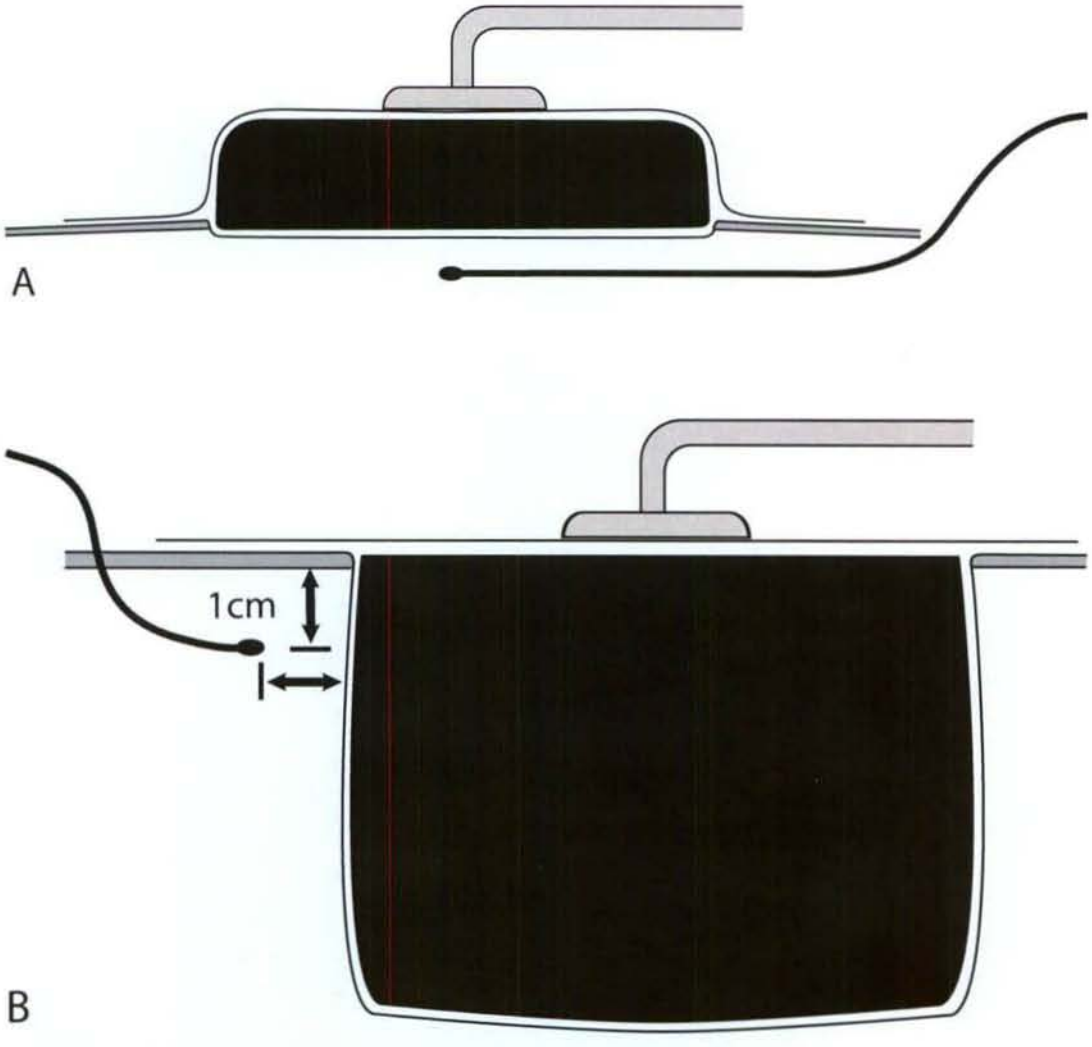

Fig. 63: Cross-sectional diagrams illustrating a NPWT dressing on a conventional wound (A) and inside a cavity (B), where the foam is essentially flush with the skin. Note sensor is within the wound tissue.

The reason for cavity wounds being placed in a category of their own was because, in this scenario, it can be envisaged that the foam will attempt to suck the walls of the cavity inward, thereby reducing the surrounding tissue pressure. This is in contrast to the previous two categories where the foam merely collapses down onto the underlying wound. Interestingly, in the study on inanimate substances (see Chapter 4), NPWT in a cavity did not reduce the pressure within the substance as was expected. Instead the substance pressure increased as the suction pressures increased.

Many of these cavity wounds had bone or very little soft tissue in the base of the wound, making placement of the sensor in the base of the wound difficult. The sensor was therefore inserted (via a puncture wound through intact skin) into one of the walls of the cavity, 1 cm away from the cavity and 1 cm deep to the surface of the normal tissues (Fig. 63b).

5.3.2.3.1. Phase 1

As with the aforementioned experiments, tissue pressures were measured for different suction pressures.

Most patients in this category were paraplegics, who were frequently rotated to prevent further pressure sores. As this would interfere with readings, Phase 2 of the experiment was not undertaken in this group. Therefore no monitoring over 48 hours was done and the sensor was removed immediately following completion of the first phase.

All patients gave informed consent to having the sensor placed and the study was approved by the Human Research Ethics Committee of the University of Cape Town (approval number: 399/2007). The only exclusion criteria were wounds in children, unconsenting adults and wounds in which NPWT was contra-indicated, according to current guidelines.[14]

5.3.3. Data analyses

The change in tissue pressures are presented as mean values with standard deviations. Data for suction pressure increments were analysed using the repeated measures of analysis of variance with pressure as a factor ($p<0.05$ regarded as significant) on the SPSS version 14 (SPSS Inc, Chicago, Ill, USA). Change in pressure with time was assessed by estimating the mean gradients (β, the standardised regression coefficient) using regression analysis. To determine whether circumferential and non-circumferential dressings had a differential effect this variable was added to the regression analysis.

5.4. Results

The sensor recorded increased tissue pressures when manual pressure was applied to the foam over the wound in all wound groups, prior to starting the experiment. This demonstrated that the sensor was detecting the appropriate pressure changes in the tissues. None of the patients developed complications as a result of the indwelling pressure sensor.

5.4.1. Circumferential NPWT dressings

5.4.1.1. Phase 1

In this dressing group there was a significant increase in tissue pressure on application of suction ($p<0.005$), with increasing suction pressures generating increased tissue pressures (Fig. 64). There were no negative pressures recorded.

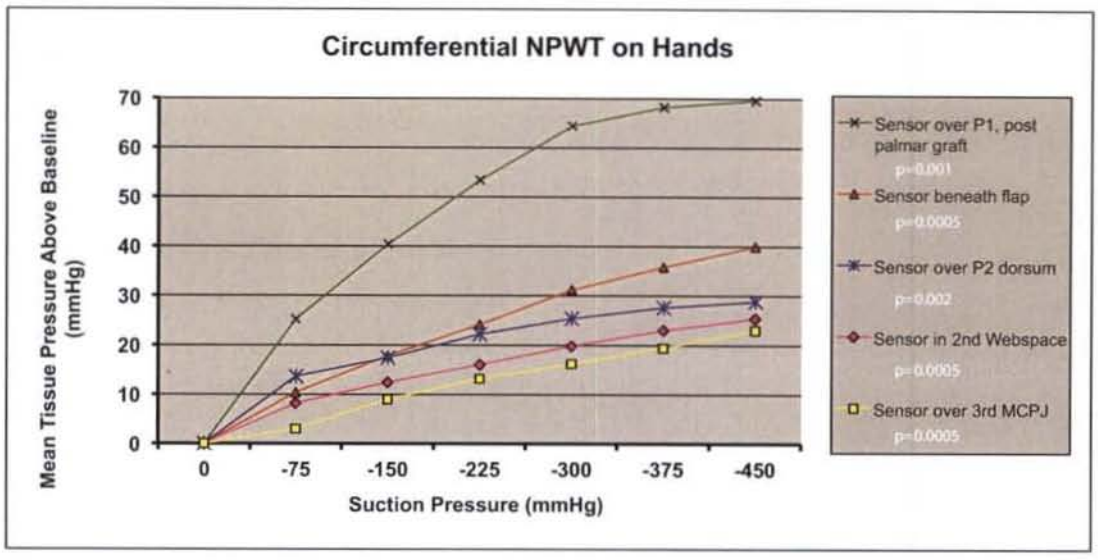

Fig. 64: Increasing tissue pressures beneath circumferential NPWT dressings in response to increasing suction pressure. P-values refer to significance of the gradients of the curves.

5.4.1.2. Phase 2

Over the 48 hour period, the increased tissue pressure decreased significantly (β = -0.21, p = 0.04) for this group. When evaluated individually, there was a significant reduction of the (increased) pressure in three of the five cases (Figs. 65 and 66, with p-values on Fig. 65).

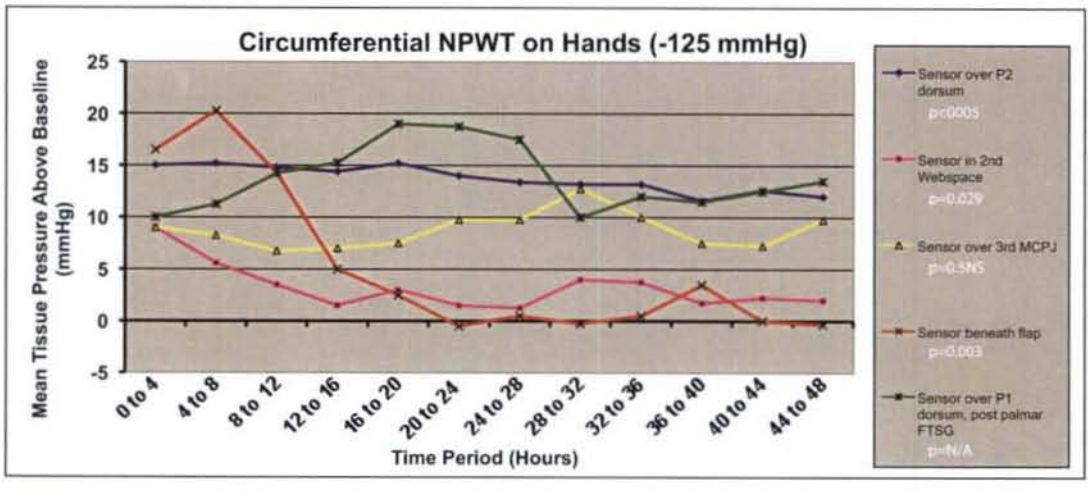

Fig. 65: Gradual decline of increased tissue pressure (above baseline) for wounds undergoing circumferential NPWT over 48 hour period. P-values refer to significance of the gradients of the curves.

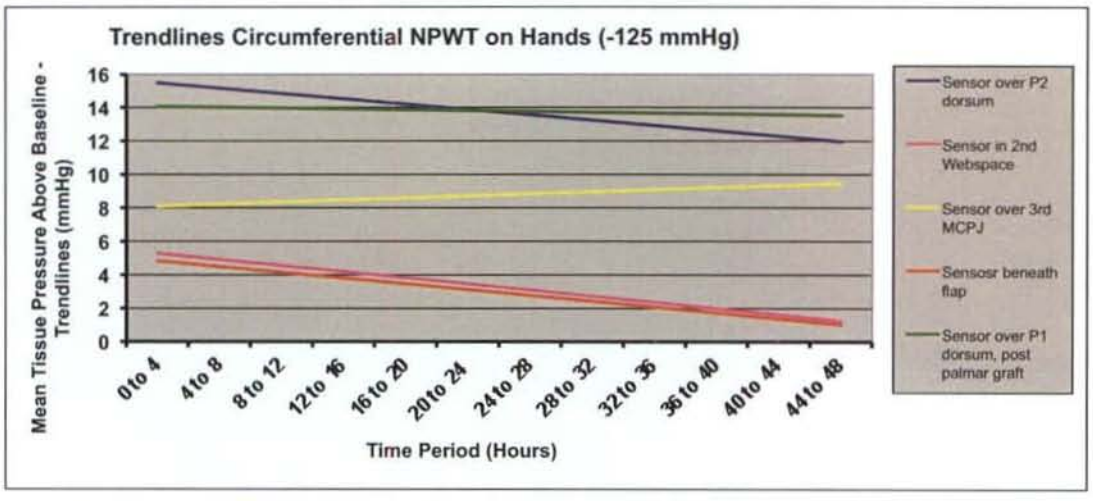

Fig. 66: Graph illustrating a trend for the increased tissue pressure (above baseline) to reduce over 48 hours during circumferential NPWT.

In one case (sensor over 3rd MCPJ) the pressure gradually increased although this was not statistically significant. One case (the hand that had the palmar FTSG) was excluded from all statistical analyses of the second phase because the suction pressure was altered during the experiment. This was due to the fact that the increased tissue pressure caused progressive pain in the patient's fingertips, resulting in the surgeon reducing the suction pressure to -75 mmHg. This was done after 28 hours and tissue pressures were seen to reduce accordingly, as did the patient's pain. The resultant trendline of the pressures in this particular case should therefore not be taken into account. Despite reducing the suction pressures in this case, however, tissue pressures gradually continued to rise.

By the end of the 48-hour period only one case (sensor beneath flap) demonstrated pressures less than the baseline pressure. In this patient, the return to baseline was reached after 24 hours.

5.4.2. Non-circumferential NPWT dressings

5.4.2.1. Phase 1

In this group too, tissue pressures increased significantly ($p<0.005$) in proportion to suction (Fig. 67). There were no negative pressures recorded.

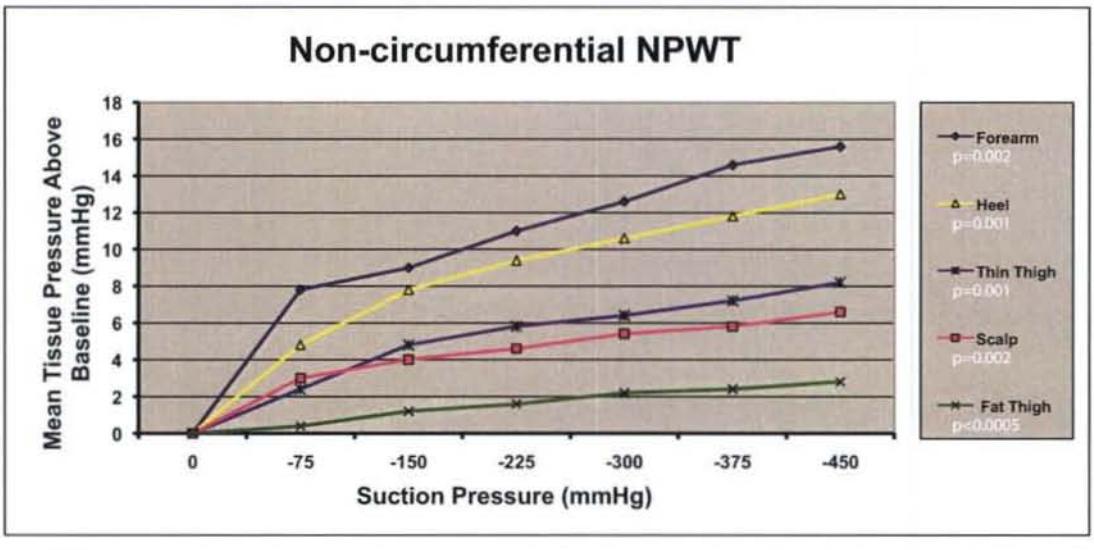

Fig. 67: Increasing tissue pressure beneath a non-circumferential NPWT dressing in response to increasing suction pressure. P-values refer to significance of the gradients of the curves.

5.4.2.2. Phase 2

For the group as a whole, there was a significant reduction of the (increased) pressure over the 48 hour period (β = -.0.494, $p<0.0005$). Individual analyses revealed that three of the five wounds had a significant reduction (Figs. 68 and 69, with p-values for 68). The reduction in the two thigh wounds was not significant. In two of the wounds (forearm and scalp) the (increased) pressure decreased to a level below the initial baseline pressure at the conclusion of the experiment. This was accomplished after 38 and 43 hours for the forearm and scalp wounds respectively.

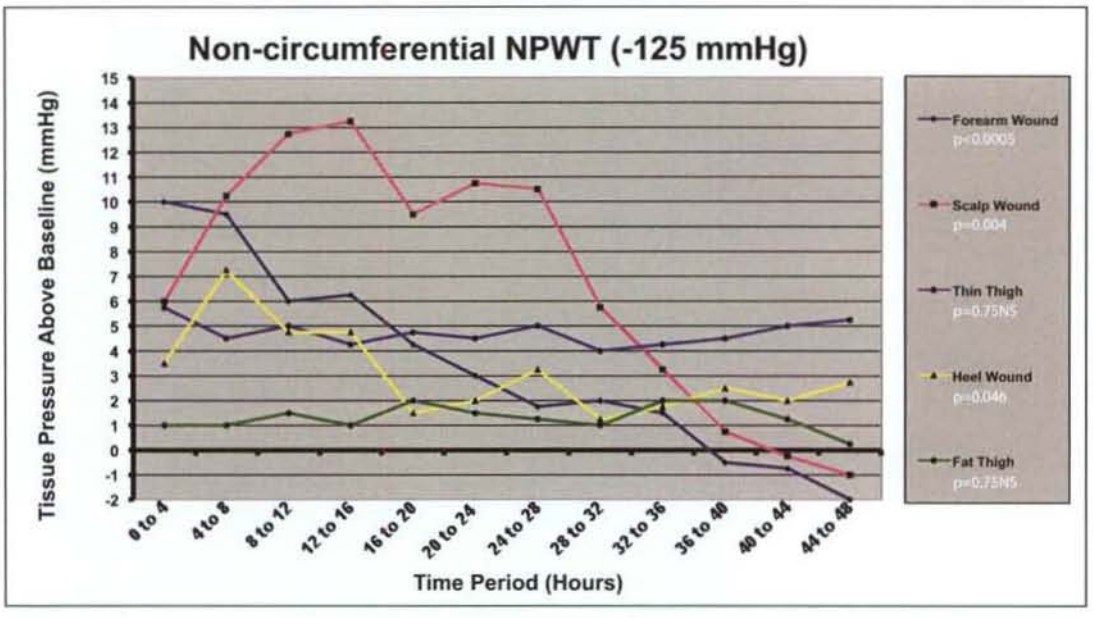

Fig. 68: Gradual decline of increased tissue pressure (above baseline) for wounds undergoing non-circumferential NPWT over 48-hour period. P-values refer to significance of the gradients of the curves.

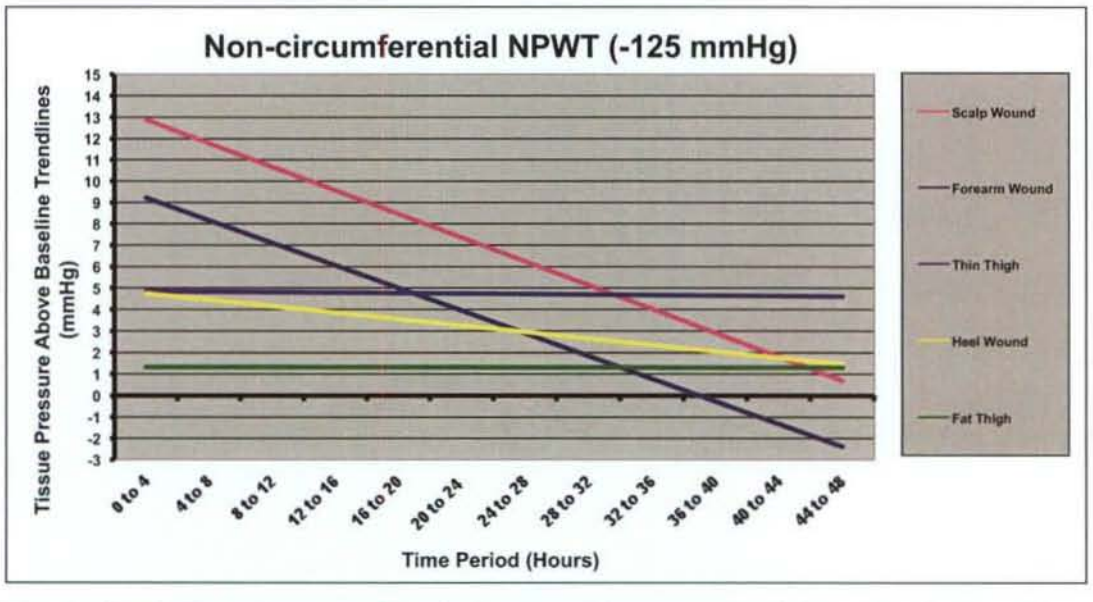

Fig. 69: Graph illustrating a trend for the increased tissue pressure (above baseline) to reduce over 48 hours during non-circumferential NPWT.

5.4.3. NPWT in cavities

5.4.3.1. Phase 1

There was a significant increase in tissue pressure in relation to suction pressure in four of the five wounds in this group (Fig. 70, with relevant p-values). The tissue pressure in the dehisced spinal wound did not increase significantly with suction (p=0.51).

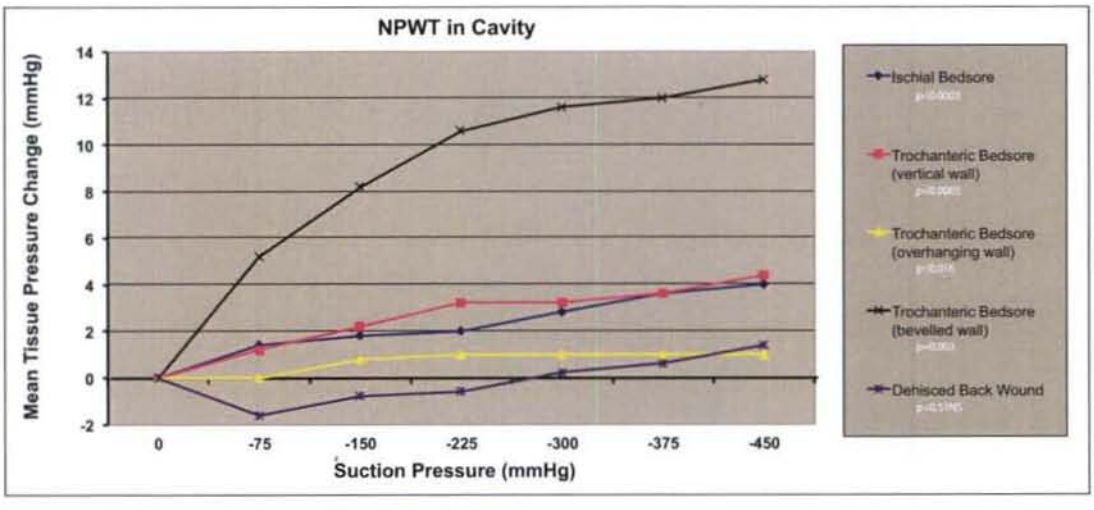

Fig. 70: Tissue pressures in the walls of a cavity undergoing NPWT increase proportionately to increasing suction pressure. P-values refer to significance of the gradients of the curves.

5.4.3.2. Phase 2

Not undertaken in this group (see Methods for reason).

5.4.4. Comparison of dressing configurations

5.4.4.1. Phase 1

The mean increase in tissue pressures in the circumferential dressing group was significantly greater than those of the non-circumferential group ($p<0.0005$) or cavity group ($p<0.0005$), although the difference between the latter two groups

was not statistically significant (p=0.269).

5.4.4.2. Phase 2

The mean reduction in increased tissue pressure over the period of 48 hours was significantly more in the non-circumferential group than in the circumferential group ($p<0.0005$), although both groups demonstrated a significant reduction.

5.5. Discussion

In all categories, the sensor demonstrated a mean increase in tissue pressure in relation to the amount of suction applied. The increase in tissue pressure was most pronounced in the wounds in the circumferential NPWT category and least pronounced in the cavity wounds.

In six of the ten wounds monitored over 48 hours, the increased tissue pressure reduced substantially with time, but only three of the ten decreased to levels below the pre-NPWT baseline pressures. The soonest this occurred was after 24 hours. In two of the ten cases, tissue pressure increased even further with time. Both of these cases belonged to the circumferential NPWT category. In three of the ten cases, tissue pressure remained 10 mmHg or more above recorded baseline pressures after 48 hours. In normal tissue, which has a capillary perfusion pressure ranging from 10 – 35 mmHg,[15] NPWT is unlikely to cause capillary occlusion. However, in tissue with severely compromised perfusion, the capillary perfusion pressures may be so low that an increment in tissue pressure of 10 mmHg (or even less) may be sufficient to cause capillary occlusion and tissue necrosis, particularly if this is continued for longer than 24 hours.

At a given suction pressure, there was a wide variation of increased tissue pressures for different wounds. This questions the recommended use a standard suction pressure of -125 mmHg on all wounds. Adapting the suction pressure to

the consistency and type of tissues, and the status of perfusion, may be a more scientific approach.

These findings represent a paradigm shift in our understanding of the physics of NPWT and conflict with the popular perception that NPWT reduces tissue pressure. A clear understanding of the physics relating to NPWT dressings should be the first step toward understanding their mechanism of action and this alternative perspective allows for new research avenues to be explored.

5.6. Conclusion

Evidence was found that increasing the amount of suction within a NPWT dressing, results in a directly proportional increase in the underlying tissue pressure in living tissues. Hypothesis (A) is therefore not rejected.

Although this was found to be most pronounced in circumferential NPWT dressings, it occurred in all dressing configurations. Hypothesis (B) is therefore also not rejected.

Although in most wounds the increased pressure gradually reduced over 48 hours, this was not always the case and some wounds demonstrated an increase in tissue pressure (albeit it not statistically significant). Hypothesis (C) is therefore rejected.

5.7. References

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6 Circumferential Negative-pressure Wound Therapy and Perfusion

Outline

6.1. Introduction

Earlier research on inanimate substances indicated that negative-pressure wound therapy (NPWT) increases the pressure within the substance to which it is applied (Chapter 4) and that this pressure increase is directly proportional to the amount of suction used. This is in keeping with earlier work by German researchers.[1, 2] The fact that this finding occurs when NPWT is applied to living tissues too was then confirmed on a study on human subjects (Chapter 5). The increased tissue pressure occurs regardless of the whether the NPWT dressing is circumferential or not. Although these findings are unequivocal, they are incongruous with the large body of evidence indicating that NPWT increases perfusion.[3-15]

The current perception is that NPWT creates a pressure gradient which draws blood toward the wound,[5, 8, 16-20] thereby increasing perfusion. This, however, implies that perfusion can be expected to be greatest at higher suction pressures and in close proximity to the foam. Yet in the study by Morykwas *et al.*,[3] tissue perfusion is reported to be decreased at pressures of -400 mmHg despite the initial increase at -125 mmHg. Furthermore, in studies from the Lund group in Sweden,[12-15] perfusion is found to be decreased adjacent to the wound edge despite being increased a few centimetres away. In contrast to both these studies, Timmers *et al.*,[4] found that perfusion continuously increased both near and beneath the foam, as suction pressure was increased , even to pressures as high as -500 mmHg.

These studies, not only contradict one another – they conflict with the basic principle that increased tissue pressure will result in decreased perfusion. A potential problem with these studies is the common use of laser Doppler. It has previously been proposed that this modality may be an inappropriate perfusion-measuring tool,[21] even more so in the context of NPWT.[22] Therefore, the need to test perfusion using an alternative modality is evident.

Although numerous studies have demonstrated that NPWT increases perfusion, the seminal work of Morykwas *et al.*,[3] is the most widely quoted.[23] Two particular pressures were highlighted in this study, with profoundly different results on perfusion. At -125 mmHg perfusion was found to increase maximally (fourfold), whilst at -400 mmHg perfusion was found to decrease to below baseline levels. Amongst others, this is one of the reasons why the most commonly used suction pressure worldwide is -125 mmHg.

Yet, as the previous two chapters demonstrated, tissue pressure is increased at all suction pressures. It would therefore be expected that NPWT will reduce perfusion at all suction pressures, including -125 mmHg. The purpose of this study was to use an alternative modality, namely radioisotope imaging, to measure perfusion and to determine whether perfusion is reduced or increased at both of the suction pressures highlighted by Morykwas *et al.*[3]

6.2. Hypotheses

Based on the findings of the tissue pressure studies the following two hypotheses were formulated:

A. "Circumferential NPWT will reduce perfusion at both -125 mmHg and -400 mmHg."

B. "Circumferential NPWT will reduce perfusion to a greater extent at -400 mmHg than at -125 mmHg."

6.3. Methods

6.3.1. Instruments and materials

Perfusion was assessed using the radioisotope, technetium pertechnetate. This

isotope has a half-life of six hours, and is one of the tracers used to assess perfusion in a wide variety of medical conditions. Following intravenous injection of the pertechnetate, a gamma camera is used to visualise the "time of arrival" and amount of radioactivity in the area being examined.

The interface dressing used to provide NPWT was polyurethane foam (Vacuum Assisted Closure (V.A.C.) Kinetic Concepts, Inc., San Antonio, TX, USA). The slabs of foam were 3.3 cm thick, 18 cm in length and 12.5 cm wide. Standard V.A.C. dressing components were used for the rest of the dressing (adhesive occlusive dressing and T.R.A.C. pad to deliver suction). As the V.A.C. pump cannot generate suction pressures in excess of -200 mmHg, a medical suction pump (Schuco, Carle Place, N.Y., USA) with a recently calibrated gauge, was used instead.

6.3.2. Subjects and technique

Ten healthy volunteers were recruited and sequentially randomised into the two different suction pressure categories. Therefore, half would receive suction at -125 mmHg and the others would receive -400 mmHg. The palm and four fingers were placed in a sandwich-like manner between two equal-sized slabs of polyurethane foam. The sandwich technique was used for the same reason as that in the pressure studies (Chapters 4 & 5); if a single slab of foam were to be wrapped around the hand, the reduction in size of this interface dressing could confound results by constricting the hand. By placing two separate slabs of foam on either side of the hand, no constriction of the hand occurs when the foam length is shortened on application of suction. The observed results are therefore more likely to represent the effects of NPWT rather than of a constricted hand.

The same was done on the contralateral hand (Fig. 71) and the dressings were completed according to manufacturer's instructions, using their adhesive occlusive drape. Both hands were then placed palm down onto the collimated

gamma camera detector. Suction was applied to one side (test hand) only and, as a control, no suction was applied to the other.

Fig. 71: Fingers sandwiched between foam dressings.

One minute after initiation of suction, the radioisotope was injected into the antecubital vein. This is a common method of injection of isotope and no reverse flow to the hand occurs. After three minutes had elapsed the gamma camera began recording the blood pool image for two minutes. This image is indicative of the total amount of radioisotope that has reached the hand via the arterial system during this time and represents the perfusion within that hand.

After the experiment, five days were allowed to pass, allowing complete excretion and decay of the isotope, which usually takes no longer than 60 hours. The

volunteers were then brought back for an identical experiment, except that this time the contralateral hand was used as the test hand and the other the control, i.e. a total of 20 hands were tested. This not only added power to the study but controlled for natural asymmetry that might occur due to hand dominance.
Each individual received the same suction pressure on both occasions (on the test hand). Any individual that demonstrated inconsistent findings in their test hands, i.e. increased perfusion in one test and decreased in the other test, would be brought back for a third scan without any NPWT dressings in place. This was to ascertain whether this finding was due to gross asymmetry of hand perfusion in that particular individual. The reason this technique was used rather than to do a pre-test scan on everyone was to limit unnecessary radiation.

A subjective assessment of the image was made by a nuclear medicine physician who was blinded to which hand had suction applied to it. An objective assessment was also carried out using the radioactivity count, which is distributed in a Poisson distribution. This activity count is the number of scintillations that the gamma camera detects and is an indication of the amount of blood that is perfusing the hand. The activity count of the area beneath the test foam was compared to the equivalent area in the control hand and expressed as a percentage of the control hand's activity count. For accuracy during statistical analyses, however, the activity counts were used rather than the percentages.

In the test hand, as the foam collapses under suction, the hand would move a centimetre or so closer to the detector of the camera on which the hand was resting. The activity count can be influenced by the distance between the object and the detector. For this reason, a double-head gamma camera was used (Siemens e.cam, Erlangen, Germany) with simultaneous palmar and dorsal acquisition (Fig. 72). The geometric mean of the two readings is then calculated to correct for distance discrepancies.

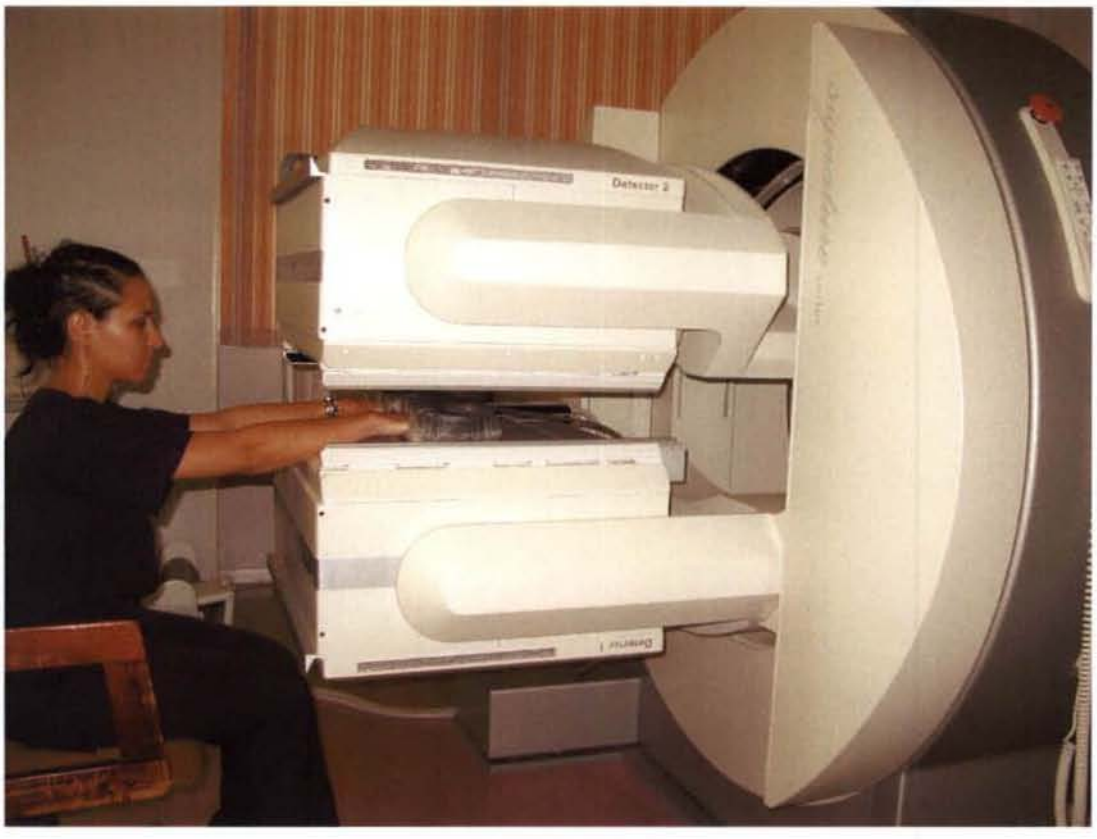

Fig. 72: Double-detector gamma camera used in this study.

6.3.3. Influence of foam density on radioactivity counts

Tests were also carried out to assess whether the foam dressing, both in its collapsed state and its normal state, had the ability to attenuate the activity count. An activity count was acquired from a vial with a known quantity of radioactive material in the absence of any dressings. The vial was then placed in the same dressings used in the study and an activity count was obtained with and without suction, at pressures of -125 and -400 mmHg respectively.

All volunteers gave informed consent to be injected with the radioisotope. The study was approved by the Human Research Ethics Committee of the University of Cape Town (approval number: 401/2007).

6.3.4. Data analysis

Each hand that received NPWT was treated as an independent set of data. Perfusion changes for each individual were analysed using the Wilcoxon test. Perfusion changes for those receiving suction pressures of -400 mmHg were compared to those receiving -125 mmHg using the Mann Whitney test on the SPSS version 14 (SPSS Inc, Chicago, Ill, USA). $P<0.05$ was regarded as significant.

6.4. Results

6.4.1. Perfusion outcomes

There were three males and two females in each of the two test groups. The mean age in the -125 mmHg group was 33 (range 29 – 36) and for the -400 mmHg group, 41 (range 31 – 64). In both the -400 mmHg group and the -125 mmHg group there was a significant mean reduction in perfusion of 40% (SD 11.5%, $p<0.005$) and 17% (SD 8.9%, $p<0.005$) respectively (Table. 2).

Table 2: Volunteer demographics and perfusion reduction for each test hand undergoing circumferential NPWT (n=20).

Volunteer No.	Sex	Age	Suction Pressure	Perfusion Reduction (%) - Left Test Hand	Perfusion Reduction (%) - Right Test Hand
1	M	35	400 mmHg	59.3	48.0
2	M	35	400 mmHg	47.8	40.5
3	M	36	400 mmHg	40.1	36.0
4	F	31	400 mmHg	45.8	26.1
5	F	29	400 mmHg	37.1	18.9
6	M	34	125 mmHg	29.0	9.1
7	F	64	125 mmHg	14.4	19.8
8	M	31	125 mmHg	30.3	19.0
9	M	42	125 mmHg	1.9	22.0
10	F	36	125 mmHg	19.5	15.1

Radioactivity counts are given in Figures 73 and 74.

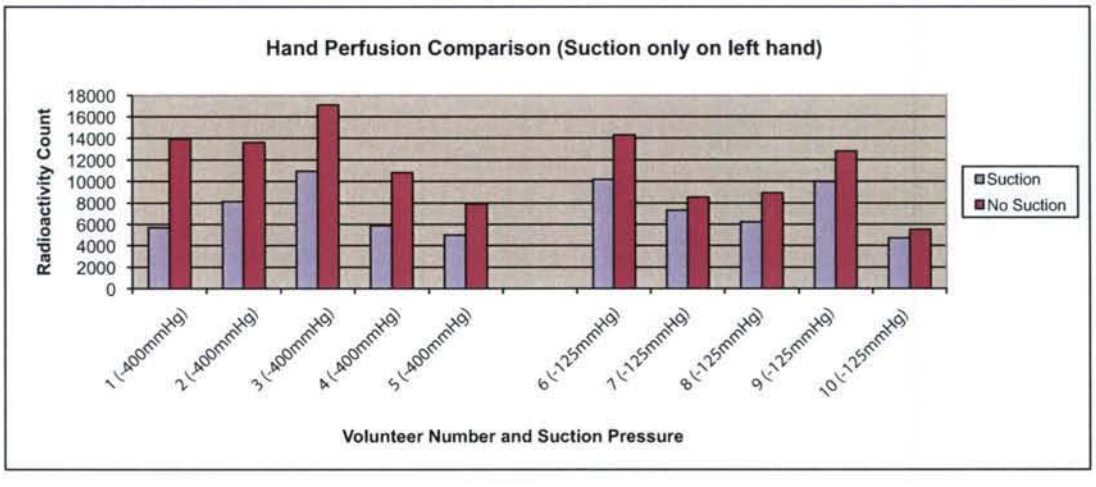

Fig. 73: Comparison of left test hand and right control hand.

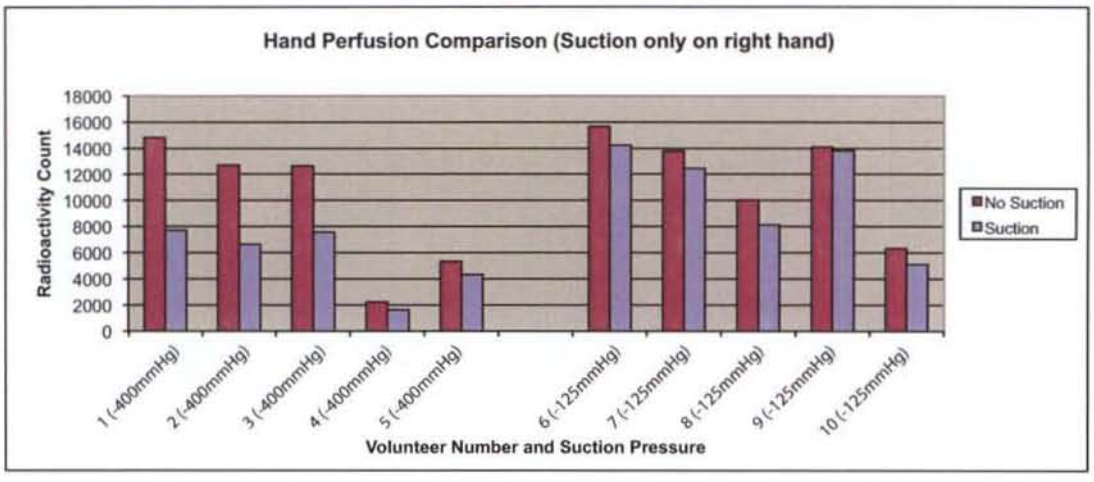

Fig. 74: Comparison of right test hand and left control hand.

The reduction in perfusion of the group undergoing NPWT at -400 mmHg was significantly greater than the group undergoing NPWT at -125 mmHg ($p<0.015$) (Fig. 74). None of the hands demonstrated an increase in perfusion.

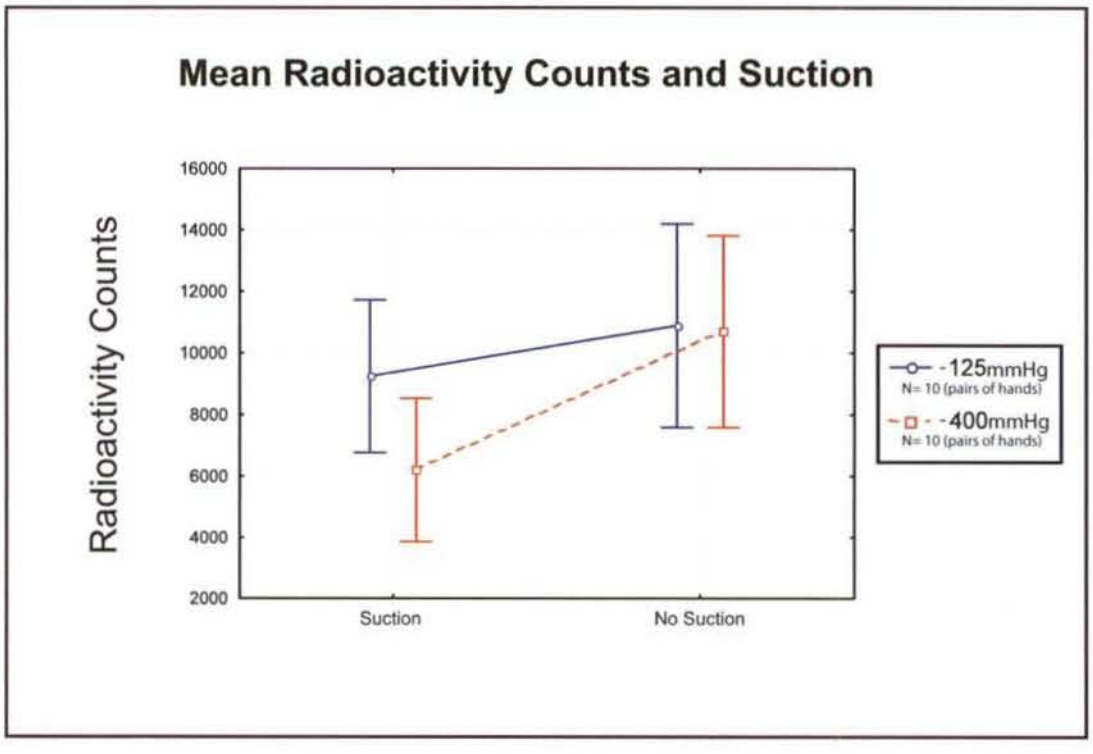

Fig. 75: Mean counts and standard deviation for both suction pressure groups. The reduction due to -400 mmHg is significantly greater than -125 mmHg ($p<0.0005$).

Typical images produced by the gamma camera are shown in Figures 76 and 77. On reporting the scans, the nuclear medicine physician subjectively identified 19/20 tests as having asymmetrical perfusion. The case reported as having symmetrical perfusion was in the -125 mmHg group.

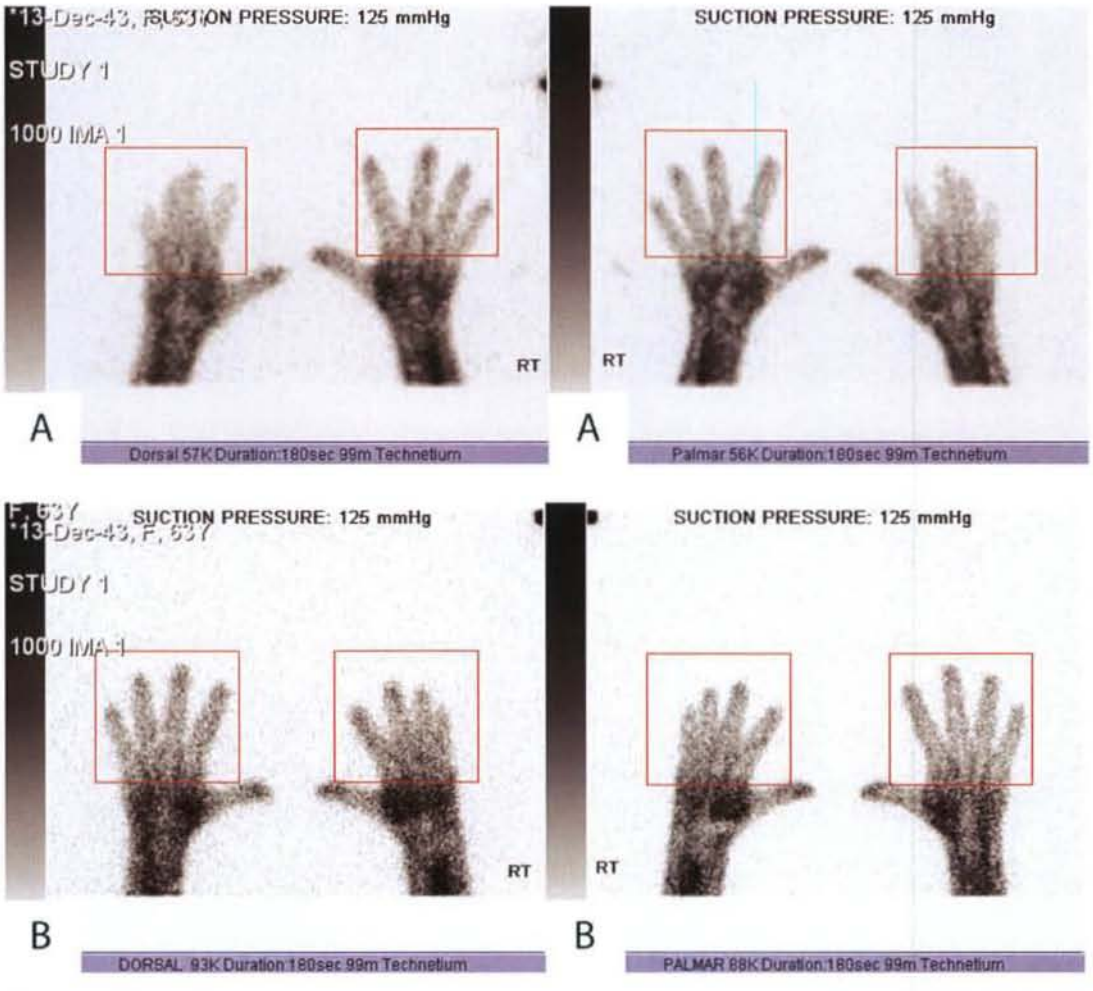

Fig. 76: Dorsal and palmar gamma camera views of both hands of a volunteer. (A) Left hand undergoing suction pressure of -125 mmHg. (B) Right hand undergoing suction pressure of -125 mmHg.

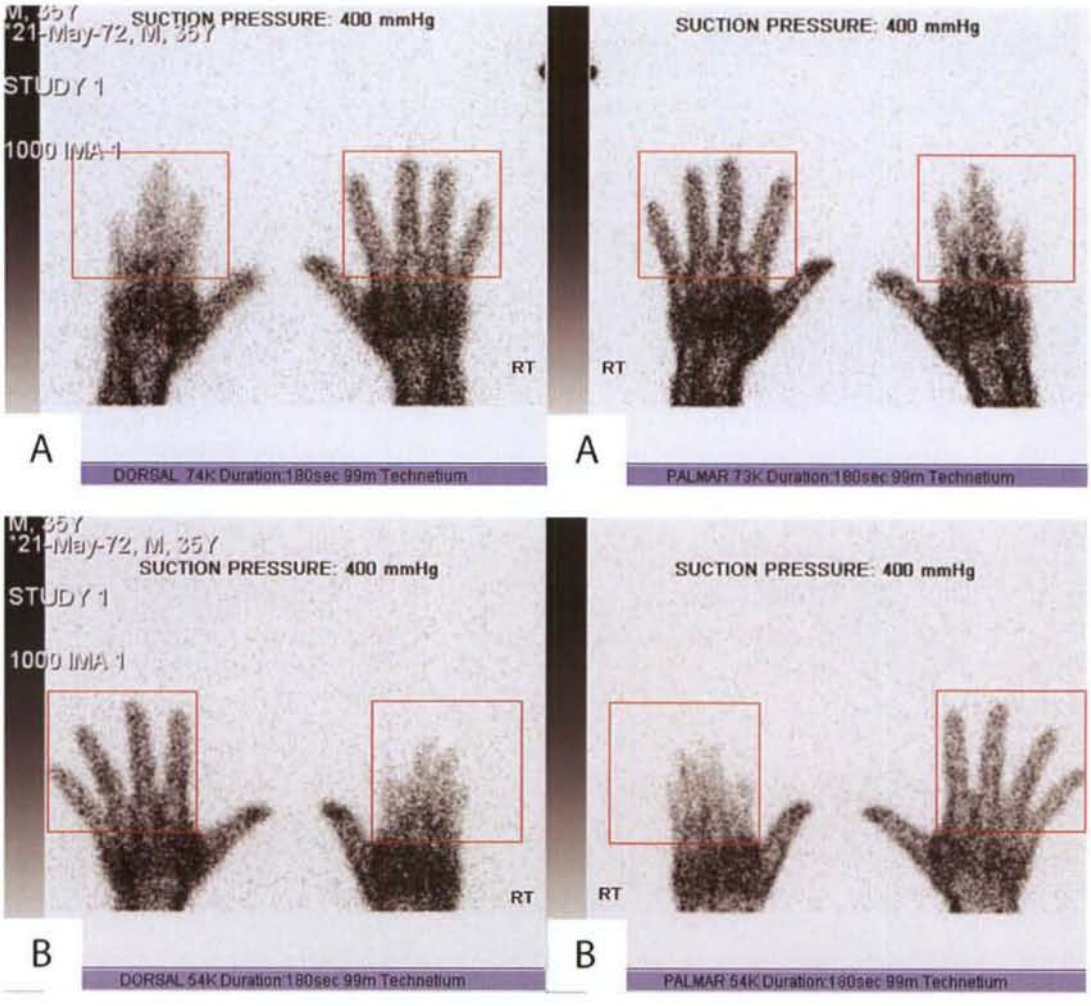

Fig. 77: Dorsal and palmar gamma camera views of both hands of a volunteer. (A) Left hand undergoing suction pressure of -400 mmHg. (B) Right hand undergoing suction pressure of -400 mmHg.

6.4.2. Influence of foam density on radioactivity counts

The presence of the foam around the test vial resulted in an attenuation of the counts by only 0.01%. Once suction was applied, this was increased to 0.9% and 2.45% for suction pressures of -125 and -400 mmHg respectively. This increase in attenuation is attributed to the increasing density of the foam as suction increases. The attenuation was factored into all calculations.

6.5. Discussion

This study demonstrated that circumferential NPWT results in a substantial decrease in perfusion and that -400 mmHg results in a greater reduction than -125 mmHg. There were no increases in perfusion. The collapsed foam affected the radioactivity count to a minimal degree but this was nevertheless factored into calculations.

These findings are in keeping with research presented in Chapter 4 and 5, where it was demonstrated that circumferential NPWT increases tissue pressure. However, this study's findings conflict with the accepted understanding that NPWT increases perfusion.

Only one study (Kamolz *et al.*) can be identified that alludes to perfusion beneath circumferential NPWT dressings.[24] Using indocyanine green video angiographics, Kamolz *et al.* demonstrated that circumferential NPWT dressings increased perfusion in burned hands.[24] However, this was assumed because of the noticeably increased perfusion that was demonstrated when the dressings were removed.

The conclusion by Kamolz *et al.* is only partly correct; NPWT did increase perfusion, but only after the NPWT dressing had been removed. This does not mean that the NPWT dressing increased perfusion whilst suction was being applied to it. It is more likely, in fact, that the increased perfusion that Kamolz *et al.* observed (when the dressing was removed) was due to the following two reasons: (1) a reactive hyperaemia secondary to the ischaemia caused whilst NPWT was being applied; (2) a reduction of oedema as a result of the increased tissue pressures. Therefore, it can be seen that although the conclusion of Kamolz *et al.* conflicts with the findings of this study, their results nevertheless compliment this study's findings.

This study's findings imply that NPWT should be used with caution on tissues with a compromised perfusion, contrary to common perception that NPWT can be used to improve perfusion. NPWT may, at a later stage, increase perfusion as oedema is reduced and after the NPWT-related hypoxia results in angioneogenesis. But this takes days to occur and is reliant on the tissues being able to survive until such time. Although most tissues can probably endure the NPWT-related hypoxia, those in which tissue perfusion is severely compromised may become anoxic and undergo necrosis before the beneficial effects occur.

6.6. Conclusion

This study has demonstrated that circumferential NPWT reduces perfusion at both -400 mmHg AND -125 mmHg. Therefore, hypothesis (A) is not rejected.

Circumferential NPWT at -400 mmHg was found to reduce perfusion to a greater degree than at -125 mmHg. Hypothesis (B) is therefore, also not rejected.

6.7. References

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7 Non-circumferential Negative-pressure Wound Therapy and Perfusion

Outline

7.1. Introduction

The research findings of Chapter 4 and 5, together with the German studies,[1,2] provide unequivocal evidence to demonstrate that NPWT increases tissue pressure, with higher suction pressure generating higher tissue pressure. This concept represents a paradigm shift in our understanding of the mechanism of action of NPWT and casts considerable doubt on the belief that applying suction will result in a pressure gradient, which will cause a surge of blood to the wound.[3-9]

The study on circumferential NPWT and perfusion (Chapter 6) demonstrates reduced tissue perfusion for increasing suction pressure, lending further support to the evolving concept that NPWT increases tissue pressure and reduces perfusion. It could be argued, however, that circumferential NPWT may actually constrict the underlying tissues, even though a sandwich technique was utilised. Indeed, it could be seen in the tissue pressure studies in Chapter 5 that circumferential NPWT generated greater increases in tissue pressure than non-circumferential NPWT did.

However, although the pressure increase of tissues undergoing non-circumferential NPWT was not as marked as those undergoing circumferential NPWT, it was nevertheless increased, without any reductions being recorded. Therefore, the question is raised whether non-circumferential NPWT will also reduce perfusion, albeit to a lesser degree to circumferential NPWT.

Prior to the research presented in the previous chapter, there were no prior studies on perfusion changes in tissues undergoing circumferential NPWT. In contrast to this, however, there is a significant body of evidence showing that non-circumferential NPWT increases perfusion[3-5, 10-19] However, as mentioned in Chapter 1, there may have been flaws in the majority of these studies, primarily related to the use of the laser Doppler as an instrument to measure perfusion. The need to test perfusion in non-circumferential NPWT, using devices which do

not rely on the principles utilised by the laser Doppler (see Chapters 1 and 9), was identified as the next step in clarifying this apparent paradox.

7.2. Hypotheses

Based on the findings of Chapters 4, 5 and 6, the following hypotheses were formulated:

"Non-circumferential NPWT will:

A: A: reduce perfusion at both -125 mmHg and -400 mmHg NPWT, and

B: B: -400 mmHg will reduce perfusion to a greater extent than -125 mmHg."

7.3. Methods

7.3.1. Instruments and materials

Unlike the perfusion study on circumferential NPWT (Chapter 6), the radioisotope perfusion technique could not be utilised in this group, as the gamma camera would be influenced by perfusion detected in tissues on the opposite side of the limb, which are not beneath the NPWT dressing. A $tcpO_2$ (transcutaneous partial pressure of oxygen) sensor (TCM30, Radiometer, Denmark), which measures tissue oxygen tension (mmHg), was therefore used in this group. Measurement of $tcpO_2$ is utilised in various fields as an indirect, yet reliable, measure of tissue perfusion.[20-22]

As with the previous studies, the interface dressing used to provide NPWT was polyurethane foam (Vacuum Assisted Closure (V.A.C.) Kinetic Concepts, Inc., San Antonio, TX, USA). The slabs of foam were 3.3 cm thick, 18 cm in length and 12.5 cm wide. Standard V.A.C. dressing components were used for the rest of the dressing (adhesive occlusive dressing and conventional K.C.I. suction tubing to

deliver suction). As the V.A.C. pump cannot generate suction pressures in excess of -200 mmHg, a medical suction pump (Schuco, Carle Place, N.Y., USA) with a recently calibrated gauge, was used instead.

The sensor was intended to measure the $tcpO_2$ of the tissues beneath the foam but logistically this is impossible. The overlying foam would apply pressure to the sensor, which in turn would apply pressure to the tissues and therefore influence readings. A doughnut-shaped NPWT dressing was therefore created (by excising a 4x4-cm portion of foam) and the sensor was placed on the skin in the middle of the doughnut (Fig. 78).

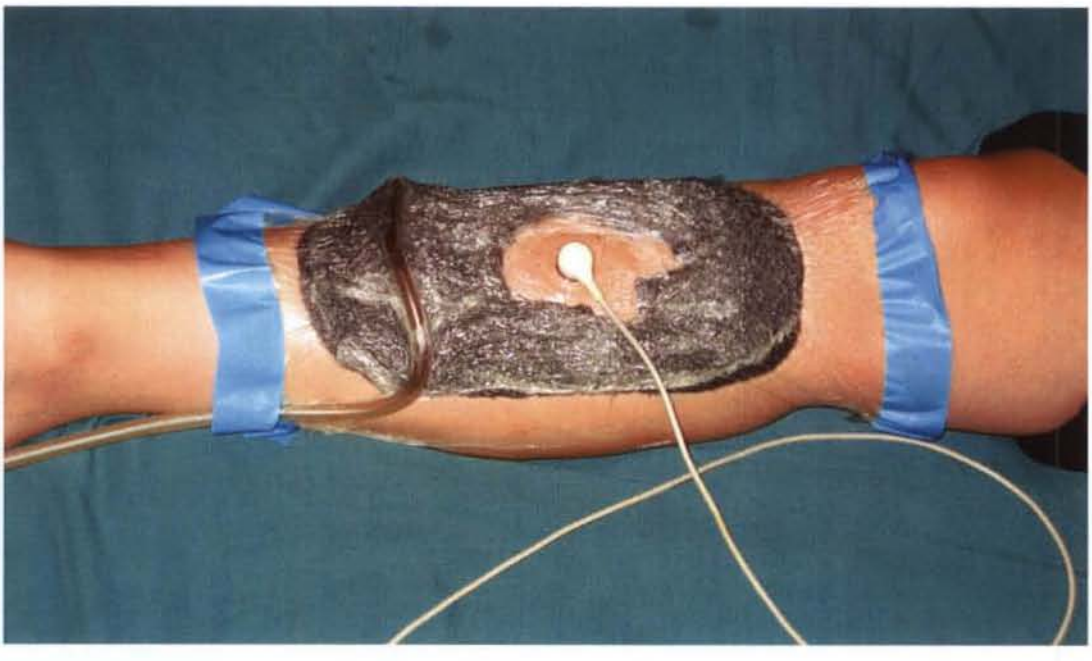

Fig. 78: Doughnut-shaped NPWT dressing with $tcpO_2$ probe on exposed central skin.

The hypothesis was that if suction increased perfusion to the skin beneath the foam, then the tissue oxygen content to the central portion of skin would also increase; if suction decreased perfusion, the opposite would occur. This was providing there was no perforator beneath the central portion of skin. If NPWT

were to result in compression of vasculature and hypoxia in the area beneath the foam, this would not influence the central portion of skin if it had its own blood supply (in the form of a perforator). For this reason, this portion of skin was tested with a hand-held Doppler (Parks Medical Electronics, Inc, Las Vegas, NV, USA) to ensure that there was no audible perforator present. If one was found, the dressing was shifted to a different location. This central skin was therefore reliant on the subcutaneous plexus of capillaries which course beneath the foam prior to reaching it.

7.3.1. Subjects and techniques

The suction dressing was placed over the anterior part of both lower legs of six healthy volunteers, i.e. a total of twelve legs were tested. Each volunteer was sequentially randomised to receive a suction pressure of either -400 mmHg or -125 mmHg. The same suction pressure was used on both legs in each volunteer and each leg was analysed independently. The experiment was carried out in a quiet room with an ambient temperature of 22° C. The individuals were sitting comfortably with both legs elevated throughout the experiment.

A consensus statement on the use of $tcpO_2$ recommended that at least ten minutes elapse prior to measuring baseline $tcpO_2$ levels.[22] Therefore, with the dressing on, a period of 15 minutes was allowed to pass in this study prior to this baseline measurements being taken. A baseline $tcpO_2$ reading was recorded every five minutes over a period of 15 minutes without any suction applied (four readings). Suction was then applied for 15 minutes, during which time readings were again taken every five minutes (four readings) with the first reading starting one minute after the suction was switched on (to allow for dynamic changes to settle). The suction was then switched off and after one minute had elapsed, readings were taken every five minutes for 15 minutes (four readings). This was continued for one and a quarter hours, allowing for one pre-suction period, two "suction on" periods and two "suction off" periods. The readings obtained during the "suction

on" periods were compared to the initial baseline (pre-suction) readings.

The study was approved by the Human Research Ethics Committee of the University of Cape Town (approval number: 400/2007).

7.3.2. Data analysis

Each leg, which received NPWT, was treated as an independent set of data. Perfusion changes for each individual were analysed using the Wilcoxon test. Perfusion changes for those receiving suction pressures of -400 mmHg were compared to those receiving -125 mmHg using the Mann Whitney test on the SPSS version 14 (SPSS Inc, Chicago, Ill, USA). $P<0.05$ was regarded as significant.

7.4. Results

The mean age of the volunteers was 34 (range 29 – 35). There were one male and two females in each group. During the period when suction was applied, there was an overall reduction in $tcpO_2$ in all twelve legs (mean 6.29 mmHg, SD 6.44, $p<0.0005$). Individual group analysis revealed that the mean of the reductions in $tcpO_2$ in the -400 mmHg group was 7.35 mmHg (SD 7.4, $p<0.0005$) and in the -125 mmHg group 5.0 mmHg (SD 4.67, $p<0.0005$). Figures 79 and 80 graphically illustrate typical examples from the -400 mmHg and -125 mmHg groups respectively, demonstrating the various time periods.

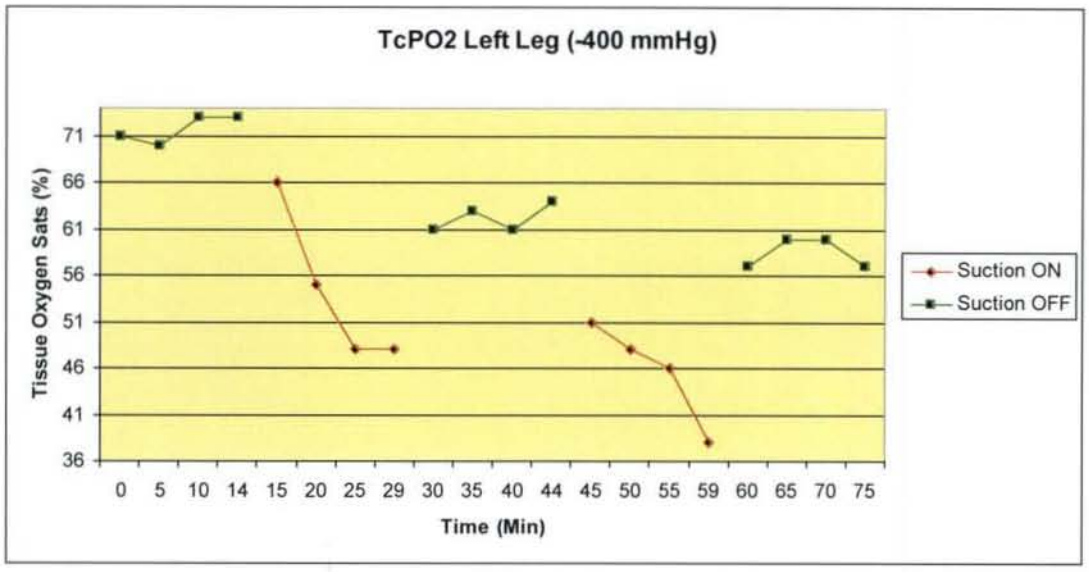

Fig. 79: Example of $tcpO_2$ fluctuations at -400 mmHg illustrating testing time periods. The readings obtained during the suction-on periods were compared to the initial baseline (pre-suction) readings.

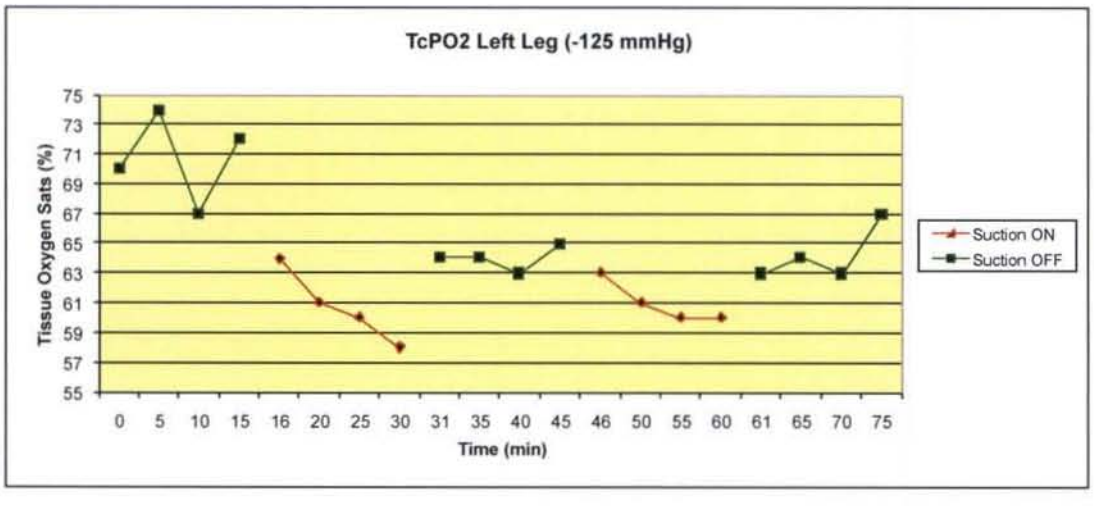

Fig. 80: Example of $tcpO_2$ fluctuations at -125 mmHg illustrating testing time periods. The readings obtained during the suction-on periods were compared to the initial baseline (pre-suction) readings.

Although there was a tendency for the mean reduction in the group undergoing suction pressures of -400 mmHg to be greater than in the group undergoing suction pressures of -125 mmHg, this was not statistically significant (p=0.07).

When the results of each lower limb were examined individually, all 12 legs demonstrated a reduction in $tcpO_2$ regardless of suction pressure (Tables 3 & 4).

Table 3: Reduction in $tcpO_2$ for each leg undergoing NPWT at -400 mmHg (n=6).

Volunteer	Leg	Suction (-400 mmHg)	Mean $TcPO_2$ (%)	Std. Deviation	Significance of Difference (p-value)
1	Left	Off	67.42	1.564	
		On	63.75	2.188	<0.0005
	Right	Off	78.50	2.646	
		On	71.00	5.182	<0.0005
2	Left	Off	64.17	5.997	
		On	50.00	8.053	<0.0005
	Right	Off	59.25	1.658	
		On	52.88	4.155	0.003
3	Left	Off	52.83	4.196	
		On	50.25	1.982	**0.083 NS**
	Right	Off	78.50	2.646	
		On	71.00	5.182	<0.0005
Combined Means	All Legs	Off	66.78	10.327	
		On	59.81	10.016	<0.0005

Table 4: Reduction in $tcpO_2$ for each leg undergoing NPWT at -125 mmHg (n=6).

Volunteer	Leg	Suction (-125 mmHg)	Mean $TcPO_2$ (%)	Std. Deviation	Significance of Difference (p-value)
4	Left	Off	66.33	3.774	
		On	60.88	1.885	0.001
	Right	Off	63.50	4.359	
		On	59.75	2.605	0.043
5	Left	Off	57.25	3.467	
		On	53.25	2.252	0.01
	Right	Off	52.50	2.876	
		On	44.88	5.817	0.001
6	Left	Off	67.25	3.079	
		On	62.75	3.615	0.008
	Right	Off	57.17	3.738	
		On	52.00	3.854	0.008
Combined Means	All Legs	Off	60.67	5.899	
		On	55.59	6.777	<0.0005

It was also noted that the means of the tissue oxygen tension after the suction was switched off for the first time (62.4 mmHg) and when it was switched off for the second time (63.3 mmHg) were both significantly less (p=0.02 and p=0.03 respectively) than the pre-NPWT mean (65.4 mmHg).

7.5. Discussion

This study demonstrates that there is a substantial decrease in perfusion beneath non-circumferential NPWT, even at widely used suction pressures of -125 mmHg, in keeping with the preceding work on tissue pressure beneath non-circumferential NPWT (Chapter 5).

A considerable amount of evidence has been presented thus far, which supports the evolving concept that NPWT increases tissue pressure and consequently reduces perfusion. These findings are particularly significant for the suction

pressure of -125 mmHg because at this suction pressure, which is the most widely used and researched suction pressure, all other studies have demonstrated an increase in perfusion.[3-5, 10-19]

These contrasting paradigms regarding perfusion may be explained by the previously raised concerns regarding laser Doppler reliability,[23, 24] which are discussed in further detail in Chapter 1 (section 1.4.4.1) and Chapter 9 (section 9.1.2). Briefly, the laser Doppler reading may be affected by movement of the dressing during the activation of NPWT and the change of the calibre of capillaries which are compressed by the dressing.

Although the body of evidence presented thus far appears to contradict prior research, it follows the logical principles of physics one would expect when tissue pressure is increased. It is counterintuitive that an increase in tissue pressure can result in an increase in perfusion, as suggested by previous research. The fact that tissue pressure is increased as a result of NPWT is not only supported by the findings presented in Chapters 4 and 5 but also those of Maier *et al.*[1] and Willy *et al.*[2]

7.6. Conclusion

Sufficient evidence was found to support the fact that non-circumferential NPWT decreases perfusion at both -400 mmHg and -125 mmHg. Hypothesis (A) is therefore not rejected.

Although there was a trend toward a greater reduction in perfusion at -400 mmHg than at -125 mmHg, it was not statistically significant. Hypothesis (B) is therefore rejected.

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8 Thermography Study

Outline

8.1. Introduction

In Chapter 5 it was demonstrated that negative-pressure wound therapy (NPWT) created a zone of increased tissue pressure around the dressing. The *in vitro* experiments (Chapter 4) showed that this zone of increased pressure extended up to 3 cm from the dressing. No zone of reduced pressure was identified to explain the observed increased perfusion reported by the world literature.[1-17]

It was subsequently demonstrated in Chapters 6 and 7 that NPWT reduced perfusion in both circumferential and non-circumferential dressings. In contrast to the world literature, there was no evidence to suggest an increase in perfusion - even at lower suction pressures.

In keeping with this, perfusion studies from the Lund group in Sweden demonstrated on numerous occasions that perfusion was indeed reduced adjacent to the wound (0,5 cm from the wound),[6-8, 10, 11] even at very low suction pressures (-10 mmHg).[7] However, a couple of centimetres further away, it was found to be increased (with a transition zone in between). At a distance of about 4 cm from the wound edge, the NPWT dressing did not affect perfusion. Therefore, if a graph of perfusion changes were to be drawn as the distance from the dressing increases, it could be envisaged to follow a sinusoidal curve, with a zone of reduced perfusion, followed by a zone of normal perfusion, extending to a zone of increased perfusion, and finally a zone of normal perfusion again (Fig. 81).

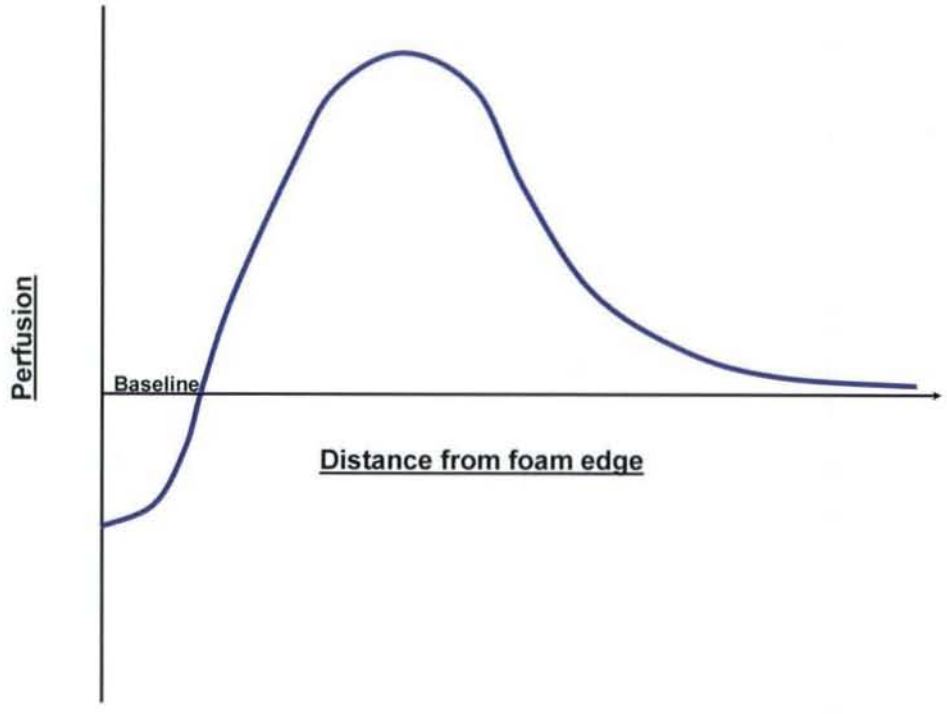

Fig. 81: Graph of anticipated pattern of perfusion changes adjacent to NPWT dressing, based on findings of research using laser Doppler.

In the *in vitro* pressure studies (Chapter 4) it was found that, although the increased tissue pressure diminished further away from the dressing, it never became hypobaric. This questions the existence of a zone of increased perfusion a couple of centimetres from the dressing, as observed by the Lund group,[6-8,10,11] increased tissue pressures do not go hand in hand with increased perfusion. Consequently a sinusoidal curve would not be expected but instead, one which starts with perfusion below baseline which then returns to baseline as the distance from the foam increases (Fig. 82).

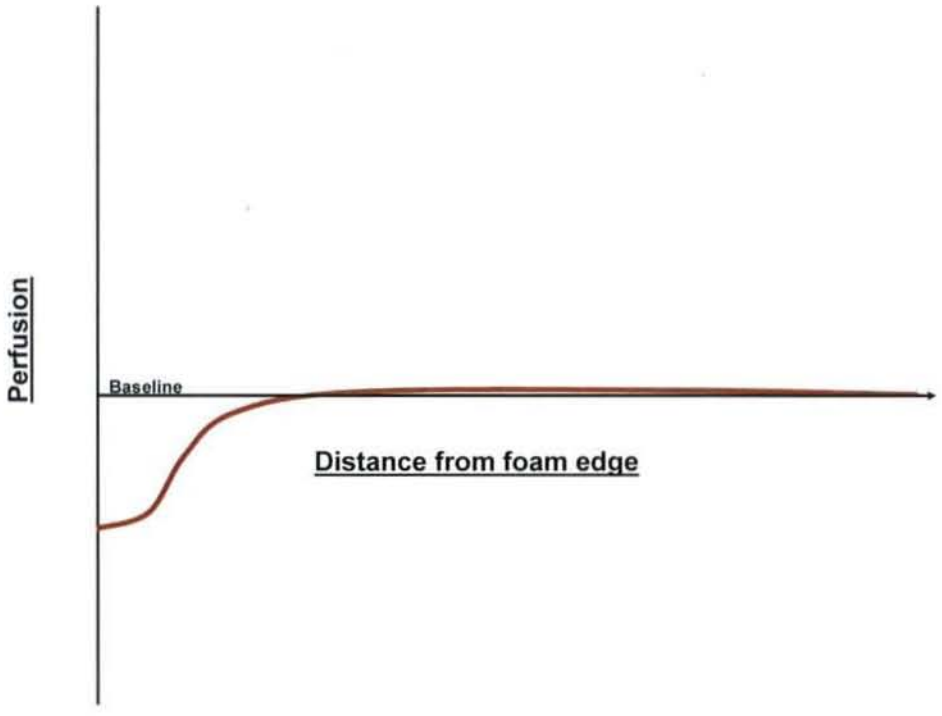

Fig. 82: Graph of anticipated pattern of perfusion changes adjacent to NPWT dressings, based on findings of tissue pressure studies.

Most perfusion imaging modalities used thus far, including the laser Doppler, can take measurements only at fixed points. A thermal imaging camera, on the other hand, allows for seamless visualisation of all the tissues surrounding the dressing and is a known technique of evaluating skin perfusion.[18-22] Its rapid detection of minor thermal changes can allow for real-time assessment of microcirculatory changes and has even been proposed as a tool for "lie-detection".[23] Thermography was used in the current study to allow simultaneous assessment of all tissue areas surrounding the dressing and, in particular, to determine whether a zone of hyperperfusion does indeed exist.

8.2. Hypothesis

The following hypothesis was formulated:

"In keeping with the fact that they increase tissue pressure, the three commonly quoted suction pressures (-75 mmHg, -125 mmHg and -400 mmHg) will all fail to result in a zone of increased perfusion within a distance of 3 cm from the dressing."

8.3. Methods

8.3.1. Instruments and materials

The interface dressing used to provide NPWT was polyurethane foam (Vacuum Assisted Closure (V.A.C.), Kinetic Concepts, Inc., San Antonio, TX, USA). The foam used was 3.3 cm thick and squares of foam with sides cut to a length of 6 cm were used. Standard V.A.C. dressing components were used for the rest of the dressing (adhesive occlusive dressing and T.R.A.C. (Therapeutic Regulated Accurate Care) pad to deliver suction). The drape was cut as a square measuring 12x12 cm and when applied, the foam was pushed flat, allowing a full 3 cm of drape to contact the skin. As the V.A.C. pump cannot generate suction pressures in excess of -200 mmHg, a medical suction pump (Schuco, Carle Place, N.Y., USA) with a recently calibrated gauge, was used instead.

A thermal imaging camera (Med-Hot MAX 110, Med-Hot Thermal Imaging, Lakeland, FL, USA) was used to capture thermal readings. The camera's thermal sensitivity (0.06°C at 30°C) and resolution (384x288) allows for a highly accurate representation of temperature variation at the skin's surface. This was mounted on a dedicated stand perpendicularly above each volunteer at a distance of 75 cm from the volunteer's back. Images were analysed using Total Vision Medical Infrared Software (Rose City, MI, U.S.A).

8.3.2. Subjects and technique

Six healthy volunteers were recruited to participate in the study. Each volunteer would have two dressings applied to their lower backs. This particular area was chosen as it is an area that can easily accommodate two dressings of this size, is fairly flat and did not require the female volunteers to undo their brassieres (Fig. 83). A flat area was desirable to minimise the potential inaccuracy that may occur as a result of varying distances from the thermal camera.

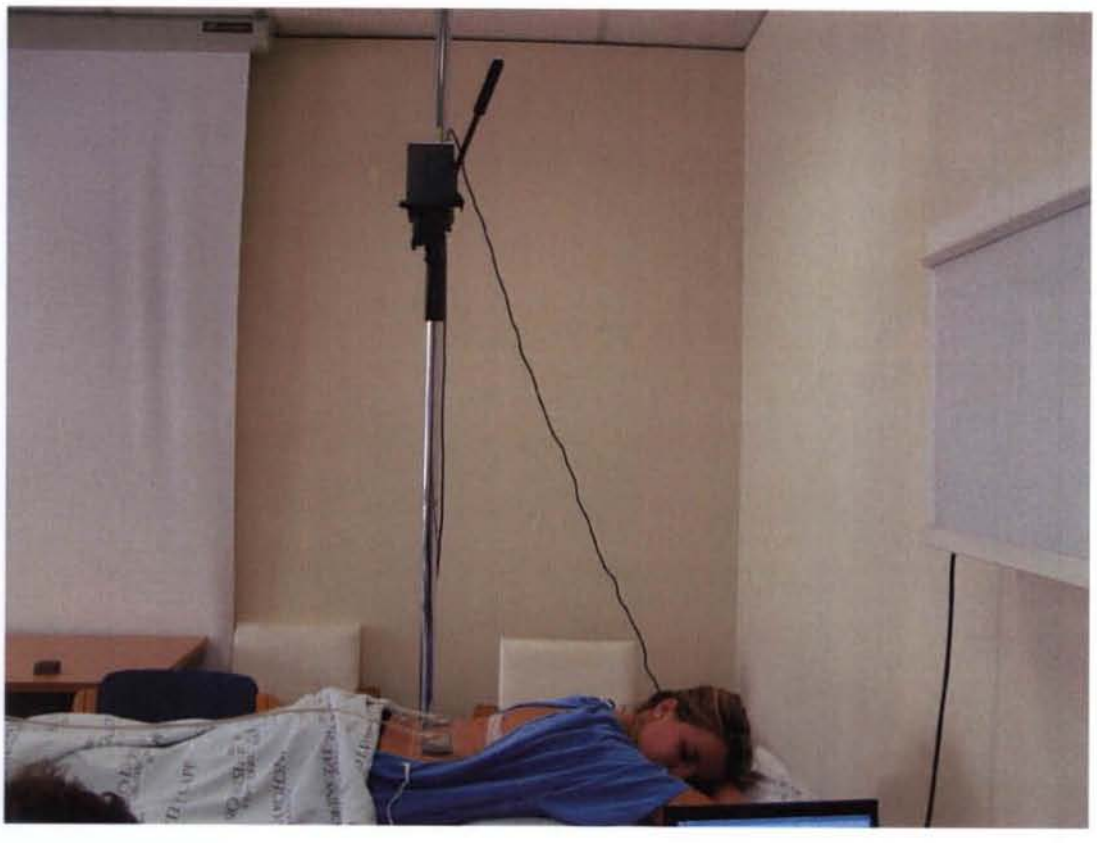

Fig. 83: Photograph demonstrating image capture set-up and position of dressings on volunteer's back.

Volunteers were sequentially randomised to three test groups, with the subjects of each group receiving the same suction pressure on either side. One group would receive -75 mmHg, the other -125 mmHg and the third group would receive -400

mmHg. The subjects were allowed to acclimatise to the room temperature (22°C) for half an hour. They were then positioned prone on an examination couch, directly beneath the thermal camera.

For each volunteer, thermal images were taken at the end of the specific phases about to be described. The phases have been named in brackets. A control image (No dressing) was taken prior to the application of the NPWT dressing. The two NPWT dressings were then applied simultaneously to the left and right side of the lower back and another image was captured one minute later (Dressing on, no suction). Suction was then switched on at the chosen suction pressure and images were taken at one minute (Suction on, 1 min) and five minutes (Suction on, 5 min) following this. A longer period of time was not desirable for this phase, as Morykwas *et al.* demonstrated that perfusion appeared to return to normal within seven minutes after suction.[1] Suction was then switched off and images were captured again at one (Suction off, 1 min) and five minutes thereafter (Suction off, 5 min).

On each image, a line was drawn from the midline of the superior edge of each foam square. The line extended to the edge of the adhesive occlusive dressing and then an equal length beyond this. The line was therefore 6 cm in length. The values expressed along this line, as the distance from the foam increases, represent temperature changes at increasing distances from the foam. The temperatures in the first 3 cm were those of the adhesive occlusive drape, the temperature of which is influenced almost exclusively by perfusion changes of the underlying skin.

The readings of the distal 3 cm were not included in calculations as this part of the skin was not covered by adhesive occlusive drape and was therefore not directly comparable to the values of the proximal 3 cm of the line. The distal readings were nevertheless recorded to ensure that there was no unforeseen obvious

increase in perfusion that occurred more distally from the suggested zone of hyperaemia (known to occur approximately 1 to 3 cm from the foam).[6-8,10,11] An obvious increase would be visible on the line graphs that could be recorded from the temperature values.

The readings in the first 0.5 cm were excluded as the adhesive occlusive drape was not tightly adherent to the skin a couple of millimetres from the edge of the foam. This could result in variations of temperature as a consequence of it not being in contact with the skin
(Fig. 84).

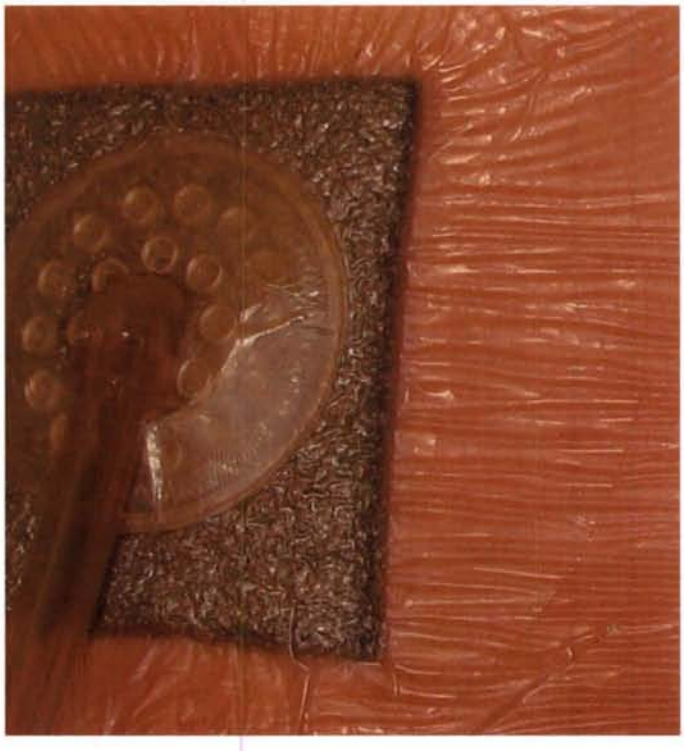

Fig. 84: Close-up photograph illustrating that the adhesive occlusive drape is raised off the skin a couple of millimetres from the dressing.

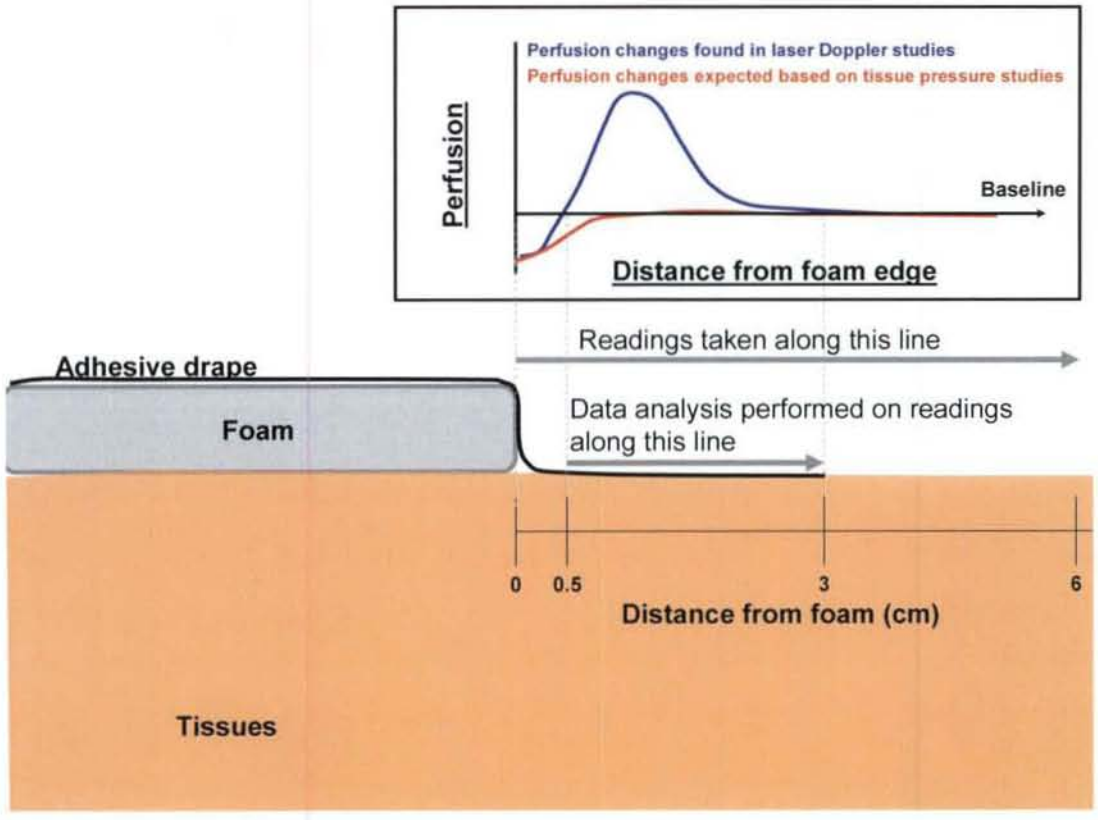

Fig. 85: Cross-sectional diagram illustrating distance over which temperature was evaluated. Only the temperatures between 0.5 cm and 3 cm were used for analysis. Note superimposed line graph to illustrate different schools of thought regarding perfusion changes. Blue line graph represents expected changes based on studies using laser Doppler to measure perfusion. Red line graph represents perfusion changes that would be expected based on tissue pressure studies.

Therefore, temperature was measured from a distance of 0.5 cm from the edge of the foam to 3 cm from the edge of the foam (the edge of the adhesive occlusive drape) (Fig. 85). For each image captured (at each phase of the study), the temperature changes along the line were plotted on a graph. The patterns of temperature change that occurred as the distance from the foam increased were compared for the different time periods.

The study was approved by the Human Research Ethics Committee of the University of Cape Town (approval number: 231/211).

Table 5: Area under the curve analysis of the different phases in the thermography experiments. There was no significant increase in temperature during NPWT to indicate an increase in perfusion 1 to 3 cm from the dressing ($F=0.47$, $df=7$, $p>0.8$)

Phase during study	N	Mean	Std. deviation	Std. error	95% Confidence interval for mean	
					Lower Bound	Upper Bound
No dressing	12	882.24	16.21	4.68	871.94	892.54
Dressing on, no suction	12	881.47	13.17	3.80	873.10	889.84
Suction on, 1 min	12	880.36	13.38	3.86	871.86	888.86
Suction on, 5 min	12	879.98	13.18	3.80	871.60	888.36
Suction off, 1 min	12	879.30	11.91	3.43	871.73	886.87
Suction off, 5 min	12	880.42	12.32	3.55	872.59	888.25
Dressing off, 1 min	12	884.51	12.82	3.70	876.35	892.66
Dressing off, 5 min	12	887.12	13.52	3.90	878.53	895.71
Total	96	881.92	13.11	1.33	879.27	884.58

A typical graph of temperature changes for the various phases, at a distance between 0.5 cm and 3 cm from the dressing, is given for the suction pressures of -75 mmHg, -125 mmHg and -400 mmHg in Fig. 86, Fig. 87 and Fig. 88 respectively. Although the temperatures beyond these distances are not shown in these figures, there were no obvious increases in temperature for any graph at distances beyond 3 cm.

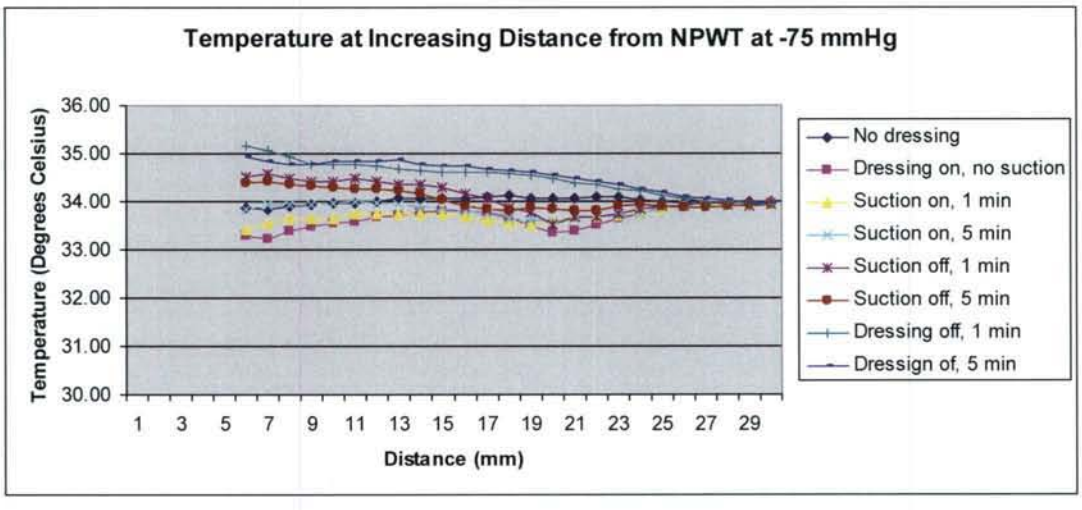

Fig. 86: Typical graph of temperature changes for the various phases, at a distance between 0.5 cm and 3 cm from the dressing, at NPWT suction pressure of -75 mmHg. Note there is no sinusoidal increase in perfusion in any of the line graphs.

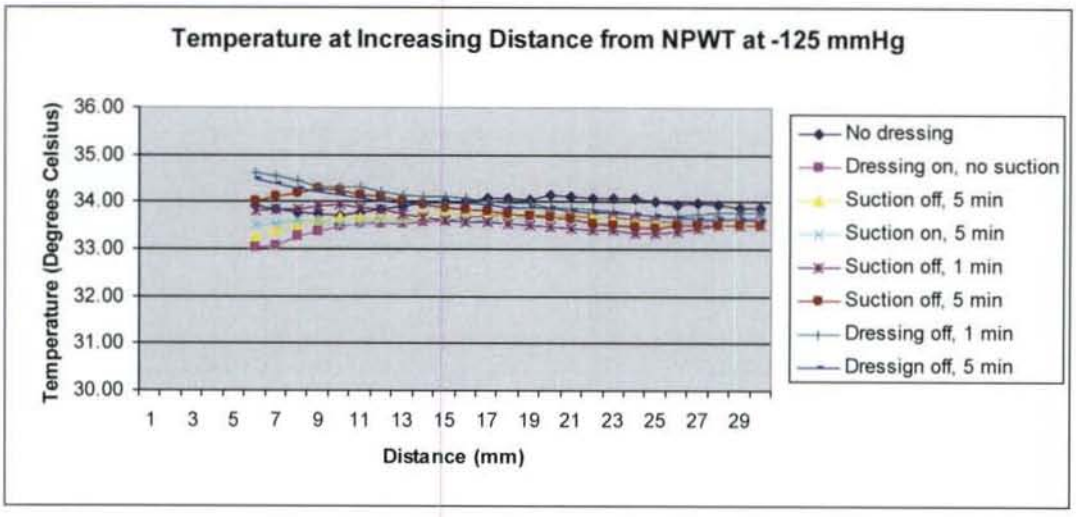

Fig. 87: Typical graph of temperature changes for the various phases, at a distance between 0.5 cm and 3 cm from the dressing, at NPWT suction pressure of -125 mmHg. Note there is no sinusoidal increase in perfusion in any of the line graphs.

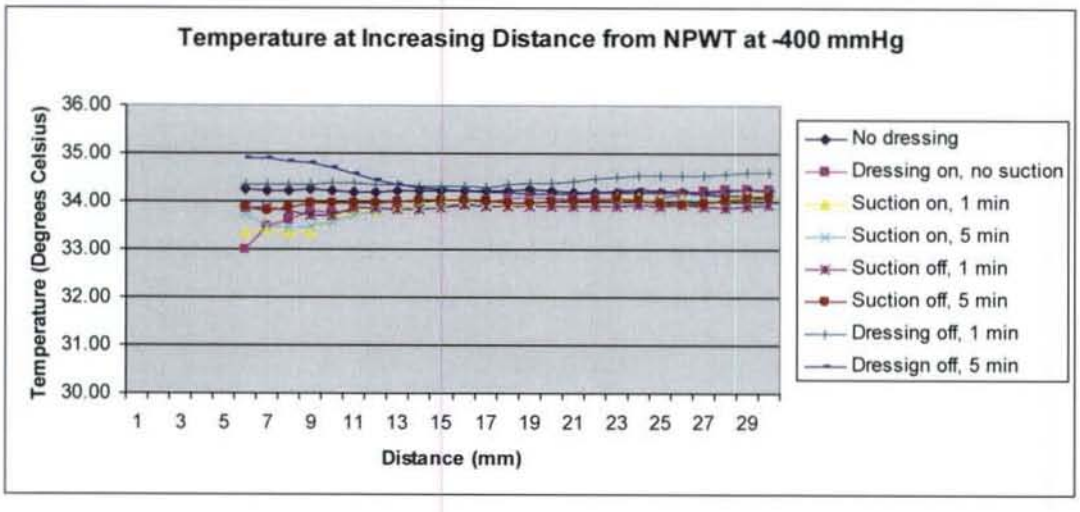

Fig. 88: Typical graph of temperature changes for the various phases, at a distance between 0.5 cm and 3 cm from the dressing, at NPWT suction pressure of -400 mmHg. Note there is no sinusoidal increase in perfusion in any of the line graphs.

There was no significant correlation between the three suction pressures tested and the difference between the mean AUC for "Dressing on, no suction" and the two "Suction on" periods (Pearson correlation = 0.24, $p>0.4$).

8.5. Discussion

The findings of this study suggested that there was no significant increase in mean perfusion (based on thermography) between 0.5 cm and 3 cm during NPWT at -75 mmHg, -125 mmHg or -400 mmHg. Furthermore, no zone of increased perfusion could be seen at any given distance beyond this. This is in keeping with previous work, that demonstrated that NPWT increased tissue pressure in the immediate proximity to the wound,[24, 25] and that no hypobaric pressures existed surrounding the dressing. The difference in temperature between the period prior to suction and the periods with suction did not correlate to the suction pressures tested, although the sample size for such a correlation may have been too small.

As this is the first time such a device has been used in NPWT research, it warrants further discussion. There are a multitude of methods to assess skin microcirculation. Thermography is based on the principle of the Stefan-Boltzmann Law, whereby the energy flux emitted by a surface is related to its temperature. All matter emits radiant energy or thermal radiation as a consequence of its absolute temperature. In human skin, the amount of radiation emitted is proportional to the change in temperature of the skin surface, which is proportional to changes in microcirculation.[18] Importantly, skin heating is dependent on both external and internal factors, such as heat delivery by blood flow and conduction properties of the subcutaneous structures. External factors such as radiation, convection, and conduction must be closely controlled in order to ensure good accuracy of thermography. Thus under thermally neutral conditions skin-surface temperature is controlled only by the blood flow rate of the cutaneous tissue.[19] Consequently changes in blood flow may be estimated by measuring temperature variation via thermography. In this study, although every endeavour was made to ensure thermally neutral conditions, this was not as crucial as in most thermography research studies because absolute values were not the desired outcomes. Instead, the changes in temperature and the pattern of the temperature change as the distance from the foam increased was sought.

Thermography has been found to be a good modality for accurately measuring skin blood flow[20] and has found utility in many other scenarios, both clinical and experimental. These include the measurement of blood flow in burns,[22] atherosclerosis,[26] peripheral vascular disease,[27] varicose veins[28] and many other disease states.[29-31] The main advantages of thermography are that it is relatively easy to use, is a non-contact device, has good spatial resolution and can map temperature distribution across regional surfaces of the body.[21] The fact that it is non-contact makes it especially useful in this study, as there is no probe or sensor which could interfere with the tissue pressure and thereby, perfusion. One disadvantage particularly relevant to NPWT research is that it cannot assess perfusion change beneath the foam. However, that was fortunately not the purpose of this study. Determining whether there was a zone of increased perfusion surrounding the foam was, in fact, the purpose.

Research to date on perfusion differences due to NPWT is characterised by a large amount of conflicting data. The seminal work of Morykwas *et al.*[1] demonstrated that NPWT increases perfusion up to fourfold at a suction pressure of -125 mmHg, during intermittent therapy. It was stated that the increase in perfusion follows a bell-shaped curve as suction pressure increases, implying that there is an optimal suction pressure (-125 mmHg), following which, increased perfusion starts decreasing again. At -400 mmHg, perfusion was decreased to below baseline levels.[1] Conflicting with this latter finding, Timmers *et al.* demonstrated that perfusion is increased above baseline levels at all suction pressures, even as high as -500 mmHg.[2] Both of the aforementioned studies used laser Doppler to measure perfusion. Ichioka *et al.* used video-imaging software with direct visualisation of the subdermal vessels adjacent to a NWPT dressing; they demonstrated that perfusion is increased at -125 mmHg but decreased at -500 mmHg;[13] the latter finding concurring with Morykwas *et al.*[1] but conflicting with Timmers *et al.*[2]

The largest volume of research on NWPT and perfusion, however, is from the Lund group in Sweden. They showed that although perfusion was increased a few centimetres away from the dressing, it was decreased close to the interface dressing (0.5 cm away).[6-8, 10, 11] The zone of hypoperfusion increased as suction pressure increased.[10, 11] At distances greater than about 4 cm there was no influence on perfusion.[7, 10, 11] Following this finding, it was proposed that the zone of decreased perfusion may serve to stimulate angiogenesis and granulation tissue formation, whilst the zone of increased perfusion provided oxygenation and nutrient supply.

Although the finding of a zone of hypoperfusion was demonstrated in numerous papers from this group,[6-8, 10, 11] it not only conflicted with those of Morykwas *et al.*,[1] Timmers *et al.*[2] and Ichioka *et al.*[13] but also with those of Chen *et al.*[12] Chen, like the Lund researchers, also evaluated perfusion 0.5 cm from the wound edge but found this to be increased at all suction pressures tested, ranging from -5 kPa to -20 kPa (-75, -113 and -150 mmHg respectively).[12] The latter findings were determined with the use of a microcirculation microscope and video-imaging software.[12]

The majority of studies that have investigated the immediate perfusion changes due to NPWT, have utilised laser Doppler velocimetry.[1, 2, 4-8, 10, 11, 14-17, 32] It has previously been proposed that this modality may be an inappropriate perfusion-measuring tool,[33] even more so in the context of NPWT.[34] This may explain the often contradictory findings of these studies.

In this study thermography did not demonstrate an increase in perfusion. It is, however, conceivable that NPWT may indirectly result in an increase perfusion at a later stage, following a reduction of oedema and the occurrence of angioneogenesis. The finding that NWPT does not directly increase perfusion follows a logical progression from the findings of Chapters 4 and 5 and other

studies[24, 25] demonstrating increased tissue pressure as a result of NPWT. It is also in keeping with the findings of Chapters 6 and 7, which demonstrated reduced perfusion in 20 hands and 12 legs respectively.

It cannot be ignored, however, that there remains a large body of evidence demonstrating that NPWT increases perfusion;[1-8, 10-17, 32] for this there must be a reason. Hypothetically, if the laser Doppler were inherently flawed in its measuring technique (when used with NPWT)[33, 34] and all prior research using this were to be discounted, to the author's knowledge there are only three studies that remain, that demonstrate increased perfusion during NPWT.[3, 12, 13] These studies will be discussed in further detail in the next chapter (section 9.5.).

A limitation of the current study is that it was conducted on intact skin and should ideally have been conducted in wounds. However, in intact skin adjacent to a NPWT dressing, Timmers *et al.* demonstrated increased skin perfusion in healthy volunteers.[2] Therefore, the fact that this thermography study was conducted on intact skin should not be seen as a reason for the observed lack of increased perfusion.

Another limitation of the study is that the changes in microcirculation may not have been sufficient to alter the temperature to a significant degree. However, based on findings of Morykwas *et al.*,[1] a fourfold increase in perfusion could be expected with NPWT. The findings of the porcine studies of Morykwas *et al.* were confirmed on intact human skin by Timmers *et al.*[2] An increase in perfusion of this magnitude would be noticeable with thermographic imaging.

8.6. Conclusion

No evidence could be found in this study to support previous publications, which suggest that NPWT creates a zone of increased perfusion a couple of centimetres from the foam. This could not be demonstrated at any of the three commonly researched suction pressures (-75, -125 and -400 mmHg). The hypothesis is, therefore, not rejected.

8.7. References

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9 Laser Doppler Study

Outline

9.1. Introduction

The findings of the prior chapters present considerable evidence to affect a paradigm shift in our understanding of the physics and biomechanics of negative-pressure wound therapy (NPWT). The concept that NPWT causes a pressure gradient, which causes blood to surge toward the wound and thereby increase perfusion has been invalidated by the previous chapters. Sufficient evidence has been presented using a variety of techniques to show that NPWT increases tissue pressure and thereby causes a reduction in perfusion, without any increases in perfusion recorded.

However, the unequivocal findings presented thus far directly contradict an overwhelming body of evidence which has demonstrated an immediate increase in perfusion.[1-17] The existence of these two contradicting bodies of research implies that, either the work presented thus far is flawed or the perfusion papers published in the literature are. It defies science that both findings can exist simultaneously and this apparent paradox has remained unexplained.

A common denominator in 14 of the 17 studies that found an increase in perfusion, is the use of the laser Doppler.[1, 2, 4-8, 10, 11, 14-18] Doubts have been cast on the accuracy of this modality, however,[19] particularly in the setting of NPWT.[20] An understanding of how laser Doppler measures perfusion changes warrants further discussion.

9.1.1. The mode of action of laser Doppler

The laser Doppler measurement of perfusion (perfusion units) is an arbitrary unit that is derived by multiplying the velocity and concentration of red blood cells within a tissue volume (less than 1 mm^3).[10, 11, 21, 22] These two parameters are determined using monochromatic light. This laser light is carried to the tissues via one optical fibre and the reflected light is received via another optical fibre, which transports it back to monitor.[19] The light entering the tissues scatters as

it encounters various types of cells and substances (proteins, keratin, melanin, haemoglobin, etc.) with varying optical properties.[19] When this light encounters a moving red blood cell it undergoes a wavelength shift explained by the Doppler effect, while the static objects in its path do little to change its wavelength.[11] The wavelength changes are, therefore, affected primarily by the concentration and velocity of red blood cells.[11] It has been suggested that the velocity parameter provides a more direct measure of physiological changes than the concentration parameter.[22]

9.1.2. Shortcomings of the laser Doppler

The laser Doppler has shortcomings, however. Its reproducibility is not always consistent; it is subject to motion artefacts; it lacks quantitative units; and there is no information on the volume of tissue sampled.[19] In addition, the flux signal recorded by a laser Doppler never falls to zero, even when evaluating vessels with no flow.[19, 23] This is likely to be the result of the Brownian motion of cells in static blood, vasomotor activity and electrical noise.[19, 23]

Due to the potential for motion artefacts, laser Doppler research ought to be undertaken with the probe completely immobile, with the only changing variable being the velocity and quantity of blood flowing through the vessels. Due to the dynamic nature of NPWT dressings, this immobility cannot reliably be achieved when suction is applied. It could be argued that the laser Doppler is, therefore, not suitable for measurement of perfusion changes in NPWT research.

In addition to this, other variables are introduced during NPWT, which are not accounted for. For example, the macrodeformation that the NPWT dressing generates is likely to change the specific area of tissue being evaluated (before and after suction is applied). Capillary beds could become more dense, as more tissue is compacted into a smaller area, thereby increasing the concentration of red cells measured (or the opposite could occur depending on whether tissues

are distracted or compressed). Furthermore, the macrodeformation may shift the focus of the probe, so that an entirely different cubic millimetre of tissue is being measured to the original one prior to the application of suction.

More importantly, however, the macrodeformation may also compress vessels, resulting in a reduced diameter. This has important implications on the dynamics of flow. The continuity equation is a principle in fluid physics, whereby it can be simply stated that fluid in steady state flow will undergo an increase in velocity if the tube in which it is flowing undergoes a reduction in diameter. Theoretically, therefore, blood attempting to pass through a narrower vessel will have an increased velocity, although not necessarily an increased flow.[24] This would be analogous to compressing the end of a garden hosepipe, which would result in an increased velocity of the water, despite a potentially reduced total flow.

As velocity is one of the parameters used to determine perfusion, this may result in the laser Doppler producing a falsely elevated value for perfusion, despite the actual perfusion potentially being reduced when vessels are compressed. However, if the capillary pressure happens to be lower than the tissue pressure generated, resulting in capillary collapse, then the laser Doppler is likely to correctly record a reduced perfusion, as velocity is likely to become zero. Therefore, in different tissues or anatomical regions, it can be expected that these seemingly opposite changes in perfusion may occur despite the same suction pressure (the tissue pressure generated in different tissues varies for a given suction pressure). In addition, the capillary pressure also varies in different tissues or anatomical regions. For all of the above reasons, it may be possible that, at a given suction pressure, the laser Doppler will record an increase in perfusion in one area, yet a reduced perfusion in another.

If this theory were true, then one ought to be able to test this by merely compressing tissue and observing how the laser Doppler responds to the perfusion changes as

a result thereof. It is common knowledge that compressing tissues would reduce perfusion and therefore the correct laser Doppler reading ought to be a reduction in perfusion, no matter how small the pressure that is being applied. If, however, the theory is correct, then gentle pressure, which would partially occlude vessels, would be interpreted as an increase in perfusion (Fig. 89). If the manual pressure were to exceed the closing pressure of the capillaries, then the laser Doppler may record a reduced perfusion (Fig. 90).

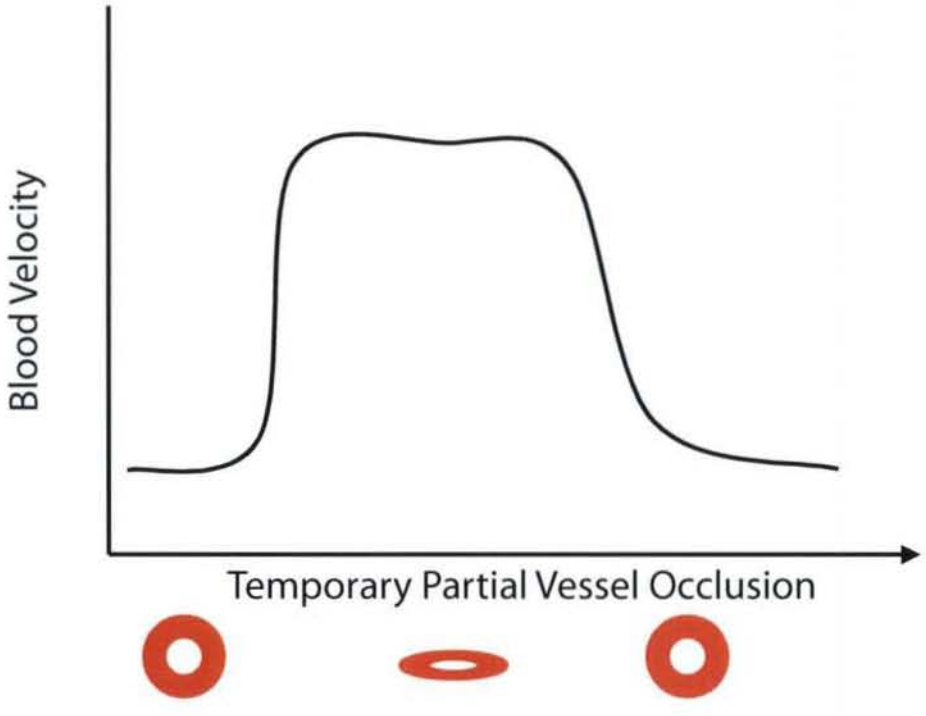

Fig. 89: An illustration depicting the theoretical change in blood velocity during a period of compression which only partially occludes the capillaries.

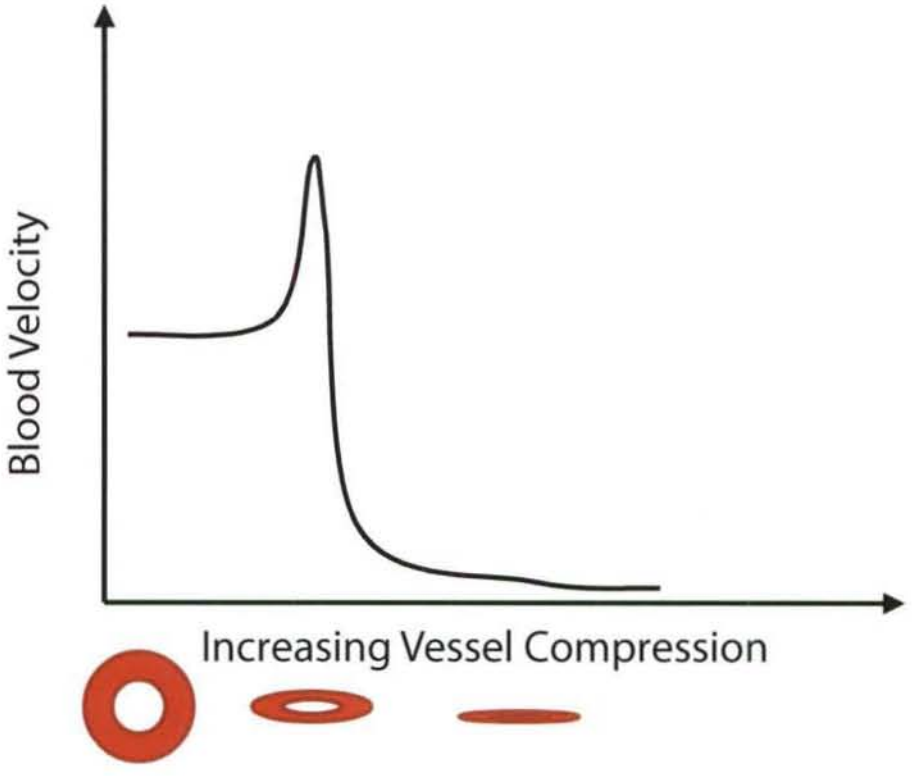

Fig. 90: An illustration depicting the theoretical change in blood velocity during compression which exceeds capillary closing pressure.

This proposed measurement flaw of the laser Doppler, particularly relevant to NPWT research, has not been investigated before. If proven to be true, this could provide clues to explain why laser Doppler studies on perfusion changes following NPWT have demonstrated an increase in perfusion. This theory was tested in the current study.

9.2. Hypotheses

Based on the theory presented above, the following hypotheses were formulated:

A: "When suction is applied at a set pressure of -125 mmHg, changes in perfusion (recorded by laser Doppler) will be different for different areas of the body."

B: "A recording of increased perfusion (by the laser Doppler) can be elicited by merely applying an external force to a NPWT dressing, despite the absence of suction."

9.3. Methods

9.3.1. Instruments and materials

A laser Doppler device (moorVMS-LDF2, Moor Instruments, Devon, UK) was used to record perfusion. The sensor was placed 1.5 cm from the edge of the foam, directly on the skin in a dedicated probe holder (Fig. 91).

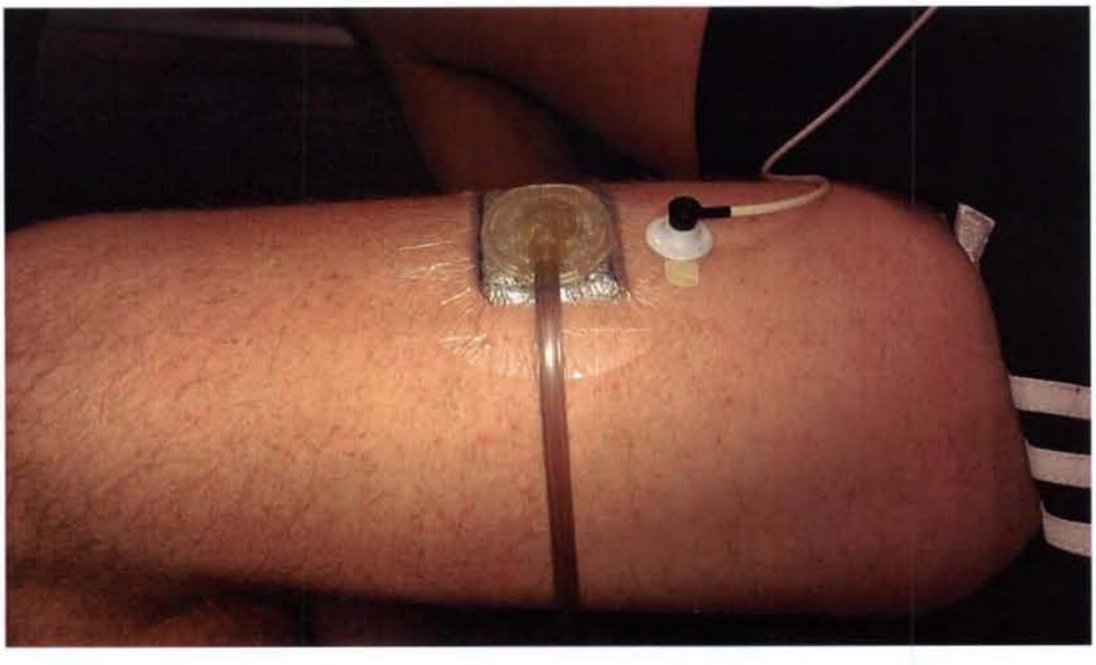

Fig. 91: Photograph depicting the position of the sensor in relation to the NPWT dressing.

The NPWT system used was the Vacuum Assisted Closure (V.A.C.) device, which uses reticulated, open-cell, polyurethane foam (Kinetic Concepts, Inc., San Antonio, TX, USA). Slabs of foam (3.3 cm thick) were cut into squares (4x4 cm).

The adhesive occlusive drape was cut to measure 8x8 cm. The manufacturer's T.R.A.C. (Therapeutic Regulated Accurate Care) pad was used to transfer the suction to the dressing. The suction pump was set to generate suction at -125 mmHg in the continuous mode.

The technique of applying manual pressure to test the hypothesis was by using two weights, measuring a total of 1.75 kg. It was initially felt that the magnitude of the weight should be determined by determining the pressure applied to the skin by the NPWT dressing. This could be done with a force sensor. However, that would only represent the vertical forces being applied to the skin and not the additional horizontal forces that a NPWT dressing appears to generate. This would therefore have been an inaccurate way to determine the amount of force (weight) to apply to the dressing to simulate the compressive forces created by NPWT. The only way of accurately determining this would be to insert a pressure sensor into the tissues. Due to the invasiveness of the latter technique, this was not feasible in the volunteers used in this study.

As there was no way of determining the exact amount of weight to apply, the weight was determined in a preliminary test, whereby different weights were applied to an identical NPWT dressing on a healthy volunteer's thigh. The least amount of weight necessary to elicit a noticeable change in the laser Doppler trace on the monitor was determined to be 1.75 kg in that experiment. As the application of more weights to the dressing was difficult to balance on the dressing, this weight was used throughout the rest of the study. It has to be mentioned at this stage that the actual weight is of less significance than how the Doppler responds to a weight.

To prevent these weights sliding off the dressing, they were strapped together using paper tape and were placed onto a light (50 g) neoprene padded band, which could be balanced on the dressing. The total weight was, therefore, 1.8 kg.

Care was taken to ensure that the neoprene band (and weights) applied pressure to the NPWT dressing only, and not the laser Doppler sensor (Fig. 92).

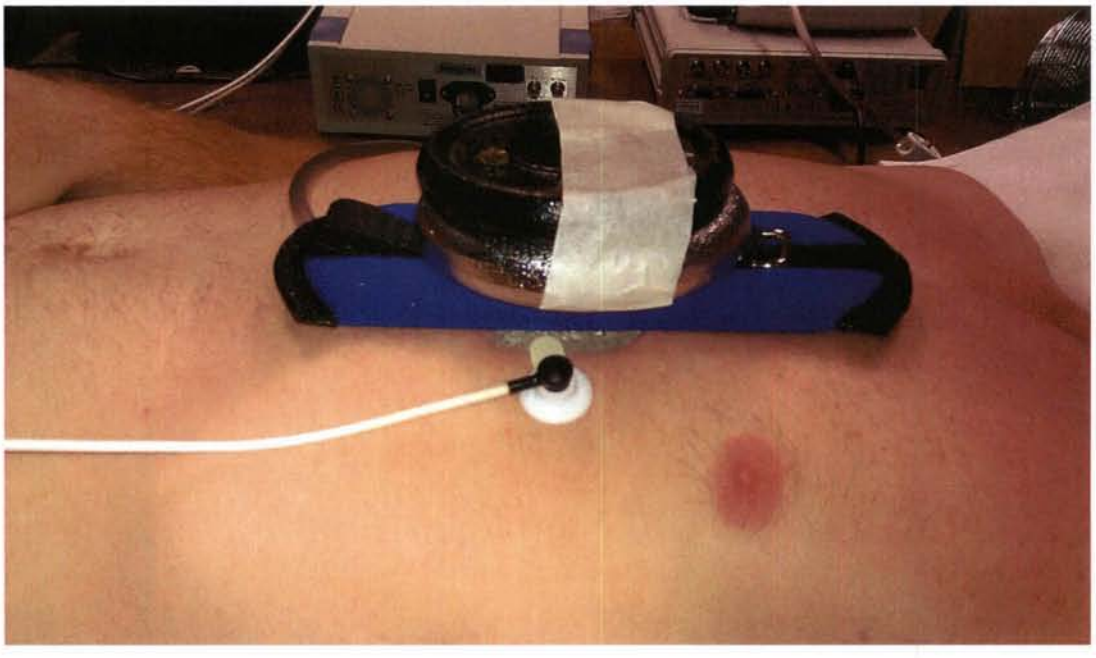

Fig. 92: Photograph illustrating the neoprene band to balance the 1.75 kg weights on the NPWT dressing. Note that no pressure is being applied directly to the sensor.

9.3.2. Subjects and technique

The experiment was conducted in a room with a temperature set at 20 °C. A total of 12 NPWT dressings were placed on different anatomical regions in two healthy volunteers. In each volunteer, a NPWT dressing was placed on their dorsal forearm (proximal third), anterior thigh and on either side of the upper chest and lower back.

The volunteers were supine during the tests on their chests, prone during the tests on their backs and sitting during the tests on their upper and lower limbs. Theoretically, if the limbs were resting on a table when manual pressure were applied to the dressing, this could result in an increase in limb pressure due to

the counter-pressure that would be applied to the limb by the table. This would potentially affect the limb and skin perfusion and therefore, during the tests on their forearms, the volunteers had their arms extended in front of them with only their wrists resting on a padded table in front of them. During the tests on the thighs, the volunteers were sitting on the edge of their seats, with their knees flexed and their feet on the ground. There was, therefore, no counter-pressure being applied to the contralateral side of the limb.

The experiment was begun in all 12 studies by recording laser Doppler readings for two minutes (with the dressing in place) to obtain a baseline reading. The following sequence was then carried out on the left thigh and chest, and right forearm and back (eight sites): suction was applied for two minutes, followed by two minutes of no suction (to allow perfusion to revert to normal) and then two minutes of manual pressure (application of a weight of 1.8 kg).

In order to ensure that the order of the sequence did not influence the results, an additional four experiments were undertaken on the contralateral sides of the chest and back, with the sequence reversed. In other words, after the baseline reading was taken, manual pressure was applied first (for two minutes), followed by two minutes without the weight, and then followed by two minutes of suction.

The study was approved by the Human Research Ethics Committee of the University of Cape Town (approval number: 340/211).

9.3.3. Data analysis

Due to the clarity of the results only a qualitative assessment was deemed necessary.

9.4. Results

In the 12 experiments, a mean of 4340 (SD 942) perfusion measurements were recorded for each 2-minute period (baseline, NPWT or manual pressure) tested. During the periods of NPWT at -125 mmHg (N=12), the mean laser Doppler perfusion recording increased in five experiments, reduced in six and remained unchanged in one (Table 6). During the period when manual pressure (weight) was applied (N=12), there was a mean increase in perfusion in six experiments and a reduction in six.

Table 6: Number of cases showing increased, decreased or unchanged laser Doppler perfusion readings following application of NPWT or manual pressure (weight).

Application of:	Laser Doppler perfusion readings			N
	Increase	Decrease	Unchanged	
NPWT (-125 mmHg)	5	6	1	12
Weight (1.8 kg)	6	6	0	12

The type of change in perfusion (increased or decreased) was the same for both NPWT and manual pressure in 10 of the 12 experiments (Fig. 93 and 94). In one of the other two experiments, the mean change in perfusion remained unchanged during NPWT but reduced during manual pressure application. In the other, the mean changes in perfusion were discordant during NPWT and manual pressure, with a mean increase being recorded during the application of manual pressure and a reduction during NPWT.

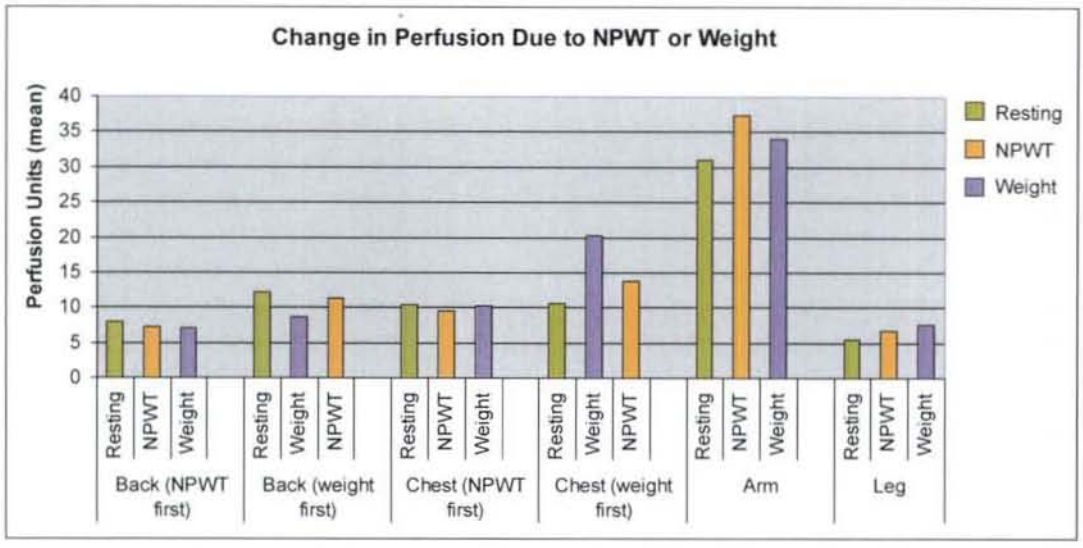

Fig. 93: First volunteer. Laser Doppler perfusion changes during various sequences of NPWT at -125 mmHg and manual pressure (weight 1.8 kg) in different anatomical regions.

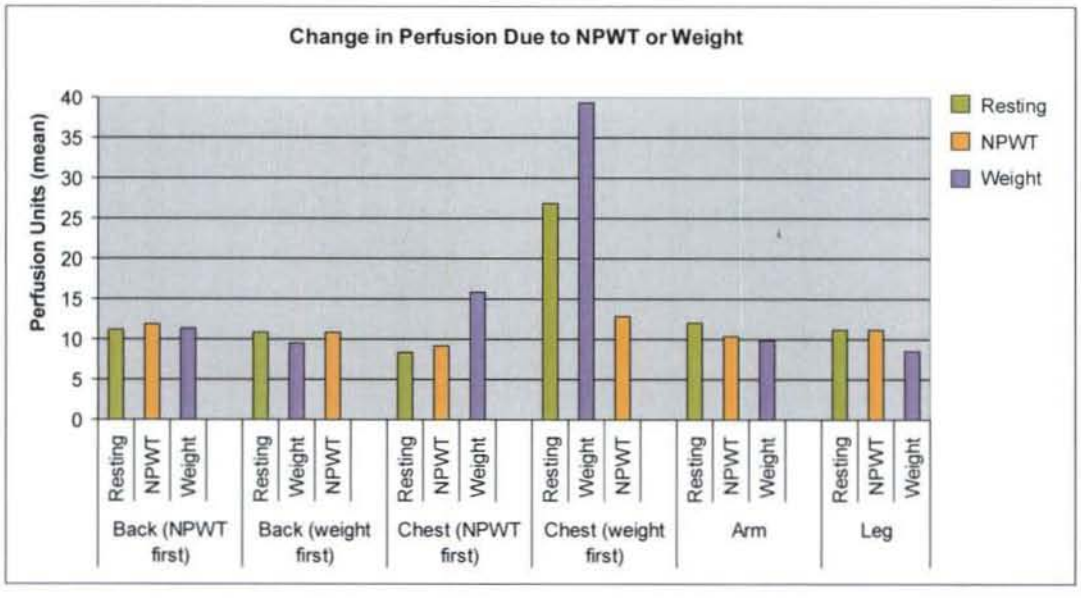

Fig. 94: Second volunteer. Laser Doppler perfusion changes during various sequences of NPWT at -125 mmHg and manual pressure (weight 1.8 kg) in different anatomical regions in second volunteer.

By virtue of the fact that the reversed sequences (n=4) had similar outcomes to the initial experiments (n=8), it did not appear that the sequence affected the outcome measurements. Only one of the initial experiments (n=8) and one of the reversed-sequence experiments (n=4) had changes in perfusion that were discordant in the NPWT and weight groups. Although a formal analysis to compare the two types of sequences was not done due to the small numbers, this aspect was of minimal relevance to the central hypotheses of the study.

9.5. Discussion

According to the laser Doppler readings, perfusion was found to be increased in some areas and reduced in others following application of NPWT, despite the use of the same suction pressure throughout. Yet the suction pressure used (-125 mmHg) is traditionally known to increase perfusion fourfold.[1]. Similar changes were seen when manual pressure was applied to the dressing without suction. These inconsistent findings are in keeping with the research presented thus far. If tissue pressure was high enough (and this varies in different tissues for the same suction pressure), then the laser Doppler would record a reduced perfusion. This represents a true reduction in perfusion and most likely results from capillaries being totally occluded, with no increase in blood velocity or perfusion within them. If tissue pressure is minimally increased but not enough to totally occlude capillaries, then the laser Doppler records an increase in perfusion due to the increased velocity in the partially occluded capillaries. The latter is not a true increase in perfusion.

Perhaps the most important observation in this study was the fact that it was possible to elicit an apparent increase in perfusion by applying manual pressure to the dressing. This was possible in half of the cases tested. This demonstrates the design flaw of this modality in NPWT research, as applying manual pressure to any living tissue should never be expected to increase perfusion. This is a

fundamental physiological principle and is the very reason pressure sores exist. This principle is the reason pressure bandages are applied to bleeding wounds. The fact that the laser Doppler could record increased perfusion in the face of tissue compression supports the hypothesis that the recordings of the laser Doppler may be misleading. In these circumstances, when vessels are compressed, the laser Doppler reading is a reflection of blood velocity rather than perfusion. And an increase in velocity can be associated with a reduction in perfusion.

A potential argument against this deduction, however, is that the manual pressure was likely to have produced a reduction in perfusion beneath the dressing, resulting in the tissue which is a centimetre or so away experiencing an increase in perfusion due to a reactive hyperaemia. If this argument were true it would be in favour of the laser Doppler being a true measure of perfusion. However, this argument is invalidated by an informal study that was previously conducted. In the latter, it was hypothesised that one could emulate the perfusion increase seen the laser Doppler traces of Morykwas *et al.*[1] (Fig. 95) by merely applying pressure to the sensor.

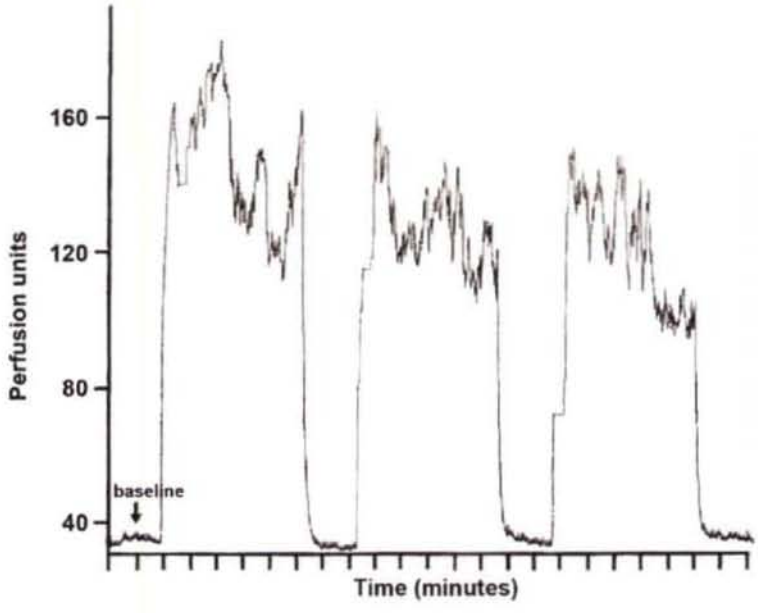

Fig. 95: Laser Doppler trace in the study of Morykwas *et al.* showing fourfold increase in perfusion during intermittent application of NPWT at -125 mmHg. (Reprinted with permission from Morykwas MJ, Argenta LC, Shelton-Brown EI, *et al.* Vacuum-assisted closure: A new method for wound control and treatment: Animal studies and basic foundation. Ann Plast Surg. 1997;38:553-562.)

A simple test was conducted where perfusion was recorded for two minutes, followed by manual pressure directly on the sensor for two minutes, and then no pressure again thereafter. It was shown that it was possible to obtain an apparent mean increase in perfusion from 7.3 (± 1.8) perfusion units to 22.3 (± 8.4) perfusion units, which then reduced to 5.6 (± 2.2) perfusion units again when the pressure was released (Fig. 96).

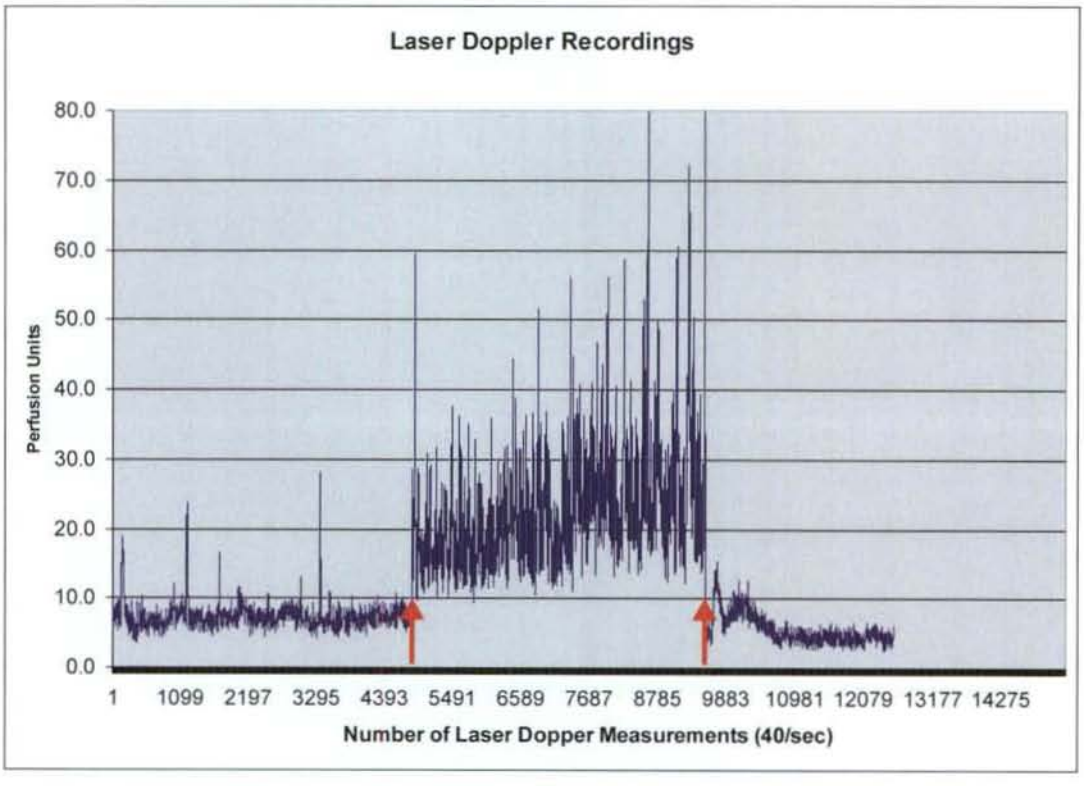

Fig. 96: Increase in perfusion recorded by a laser Doppler during application of manual pressure to the sensor probe (two minute baseline, two minute pressure, 85 seconds no pressure). Two arrows indicate the points where manual pressure was applied and released respectively.

In the latter experiment, the increase in perfusion cannot be explained by the aforementioned argument relating to hyperaemia. Tissues being compressed directly beneath the sensor cannot be hyperaemic during the time of compression, as this would imply that they have a higher perfusion than prior to compression. The fact that it was possible to elicit an apparent increase in perfusion (according to the laser Doppler) when direct manual pressure was applied directly to the sensor supports the theory that the increased perfusion observed is due to vessel compression with increased velocity, rather than hyperaemia.

Another potential argument against the hypothesis that the laser Doppler is giving misleading data, is that when manual pressure was applied to the dressings, the capillary blood may have been forced away from the skin beneath the dressing (like water being forced out of a wet sponge if pressure were applied to it). This

increase in flow away from the dressing would be recorded as an increase in perfusion by a laser Doppler probe next to the dressing, as the laser Doppler does not distinguish direction of flow. This would nevertheless be a true increase in flow (albeit it in the opposite direction) and could explain the findings of the manual pressure studies (where an increase in perfusion was recorded).

However, if this was the reason for the recorded increase in perfusion following manual pressure to the dressing, rather than the hypothesis of vessel compression (with increased velocity), this would not explain why perfusion could be shown to increase when manual pressure was applied directly to the probe in the informal study. Only the hypothesis presented in this study relating to increased velocity can adequately explain that finding, and all of the other observations.

In essence, the outcomes of this laser Doppler study invalidate all prior studies on NPWT and perfusion that utilised laser Doppler. This does not imply that these studies were poorly designed or of a low standard but merely that these researchers were misled by this device. The fact that blood velocity has been misleading researchers using laser Doppler explains many of the findings of these studies. For example, the Lund group found that perfusion is reduced in close proximity to the NPWT dressing yet increased a couple of centimeters away.[6-8, 10, 11] The capillaries in close proximity to the NPWT were likely to be totally occluded, resulting in the laser Doppler correctly interpreting this as a reduction in perfusion, whilst those a couple of centimeters away are partially occluded, resulting in the laser Doppler incorrectly recording an increased perfusion in this area. This chapter's findings also explain why the Thermography Study (Chapter 8) could not demonstrate any zone of increased perfusion around the dressing, as thermography is not influenced by blood velocity.

Furthermore, the fact that the zone of hypoperfusion that was seen in the studies of the Lund researchers[10, 11] increased in size when suction pressure

was increased, is also in keeping with the findings of this chapter. As suction increased, so too did tissue pressure, resulting in a larger zone of totally occluded capillaries surrounding the dressing. In these totally occluded capillaries, there was no blood velocity as perfusion had ceased and, as such, the laser Doppler correctly recorded a reduced perfusion. It must be pointed out at this stage that the proposed "totally occluded capillaries" might only be those being measured in the upper 1 mm^3 by the laser Doppler, while others deeper down may still be perfusing. This explained why tissue necrosis is not readily observed during NPWT.

The outcomes of this study also explain the conflicting findings that have been published regarding what the optimal suction pressures are. Morykwas *et al.* demonstrated that perfusion is maximally increased at -125 mmHg and reduced at -400 mmHg.[1] Timmers *et al.*, on the other hand, demonstrated that perfusion is increased with suction pressures as high as -500 mmHg.[2] Morykwas *et al.* were experimenting on pigs,[1] whilst Timmers *et al.* conducted their study on the forearms of healthy volunteers.[2] The different consistency of porcine and human tissue is likely to result in different degrees of capillary compression for the same suction pressure, resulting in the very different perfusion readings of the laser Doppler in these studies.

The laser Doppler's flawed measuring technique (when used in the setting of NPWT) has misled researchers since the popularisation of NPWT in 1997.[1] It was not merely giving inaccurate values – it was demonstrating changes in perfusion that were the opposite of what actually occurred. This may explain why the exact mechanism of action of NPWT has yet to be understood. Studies that were conducted to investigate the cause for increased perfusion as a result of NPWT were based on the findings of the laser Doppler studies.

More importantly, the grossly incorrect values of the laser Doppler have given rise to proposals for indications that may be dangerous or even life-threatening. The proposal that NPWT may be beneficial to ischaemic myocardium[3-5,14,17] for example, needs to be cautiously reviewed. The potential for disastrous consequences in this scenario is a reality if myocardium with borderline perfusion were rendered anoxic by the compressive effects of NPWT.

It must be mentioned, however, that there are studies where modalities other than laser Doppler were used to measure perfusion changes due to NPWT and where an increase in perfusion was demonstrated.[3, 12, 13] However, in light of our better understanding of the biomechanics of NPWT, there may be potential flaws in the methodology of these studies and these need to be highlighted.

In the study of Lindstedt *et al.* it was demonstrated that coronary artery blood flow is increased by NPWT using electromagnetic flow meter probes.[3] However, the probes were place around the roots of three coronary arteries, which were not directly beneath, or in close proximity, to the NWPT dressing. These areas of measurement were, therefore, not exposed to the compressive forces of the foam; on the contrary, they were exposed to the hypobaric pressures that were created within the chest cavity.[25] This may be an explanation for the observed increased perfusion in these vessels.

In the study of Ichioka *et al.*[13] a very superficial wound was created on the gluteal region of a mouse, allowing the intact subdermal vascular plexus to be directly visualized with an "intravital microscope". The foam of the NPWT was significantly smaller than the wound and was applied to the intact tissue adjacent to the wound, whilst the wound itself was left covered only with the transparent adhesive drape (Fig. 97).

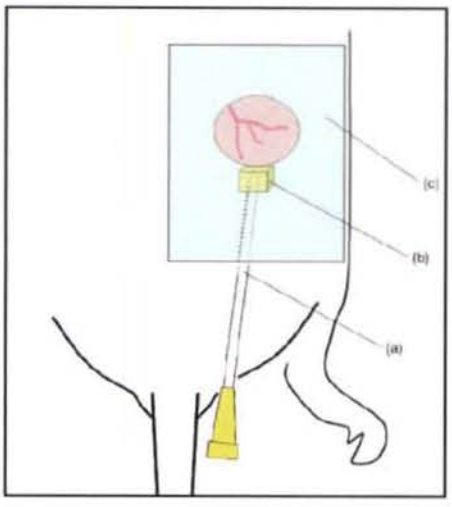

Fig. 97: The mouse model that Ichioka *et al.* used to evaluate perfusion changes due to NPWT using direct visualisation. (Reprinted with permission from Ichioka S, Watanabe H, Sekiya N, Shibata M, Nakatsuka T. A technique to visualize wound bed microcirculation and the acute effect of negative-pressure. Wound Repair Regen. 2008;16:460-465.)

The small size of the foam and its relative distance from the vasculature being observed reduced the likelihood that any compressive forces would be transmitted to these vessels or reduce their flow. The contraction of the foam may, however, have been enough to stretch the surrounding tissues, including those within the wound, thereby enlarging their calibre and increasing their flow.[13] This dressing configuration is not representative of a typical NPWT dressing on a wound, nor does it possess the biomechanical properties of a dressing comprising a relatively larger portion of foam; this may account for the findings of increased perfusion.

Chen *et al.* used a microcirculation microscope to demonstrate increased perfusion adjacent to wounds created on rabbit ears.[12] Unlike the previous two studies, this was a true representation of a typical wound within soft tissues undergoing NPWT. An increase in perfusion was demonstrated 0.5 cm from the wound edge at pressures as high as 150 mmHg. In the context of the current study the findings of Chen *et al.* are difficult to explain. The following explanation may be considered, however. In a typical wound undergoing NPWT, the depth is

sufficient to allow the foam to apply force to the outer walls of the wound resulting in increased tissue pressure adjacent to this, as demonstrated in Chapters 3, 4 and 5. Creating a wound by removing the thin skin of a rabbit ear results in a wound so shallow that there is very little, if any, outer wound "wall" of the wound cavity for the foam to apply force to. Therefore, very little pressure will be transmitted to the tissues 0.5 cm away from this contact area. As in the study of Ichioka *et al.*[13] the contracting foam and its resultant stretching of surrounding vasculature may result in increased perfusion, with the miniscule forces applied to the wound walls doing little to counteract this. This may explain the observed increase in perfusion in that study.

9.6. Conclusion

Application of NPWT at the same suction pressure (-125 mmHg) to different areas of the body resulted in different changes in laser Doppler perfusion readings. Hypothesis (A) is therefore, not rejected.

Despite the absence of suction, it was possible to demonstrate an apparent increase in perfusion in up to half of the cases by applying an external force (weight) to the NPWT dressing. Hypothesis (B) is, therefore, also not rejected.

Sufficient evidence has therefore been demonstrated in this study to suggest that, in the setting of NPWT, the laser Doppler is a flawed device for measuring perfusion.

9.7. References

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10 The Influence of Manufacturers on Negative-pressure Wound Therapy Research

Outline

10.1. Introduction

Negative-pressure wound therapy (NPWT) has been adopted by numerous surgical disciplines, some even suggesting that it forms part of the reconstructive ladder.[1] It has become one of the most successful forms of wound therapy in recent decades. In fact, the second and third most frequently cited papers in plastic surgery literature in the last 50 years, are studies on NPWT.[2] Its rapid acceptance by physicians the world over is contrasted by the slow progress researchers are making in elucidating its mechanism of action. Numerous theories have been proposed,[3-13] many of which are supported by peer-reviewed publications. However, there is a considerable amount of conflicting evidence amongst these studies and, as such, researchers continue to try and understand the workings of this revolutionary therapy. The findings in the previous chapters have clarified some of the potential causes for the conflicting findings.

Although a form of NPWT was already described by Svedman in 1979[14] and in the Russian literature in the early 80's,[15-27] the two most common techniques used today are those described by Morykwas and Argenta *et al.*[3, 4] and by Chariker *et al.*[28] The intellectual property of the technique and patented system described by Morykwas and Argenta *et al.* in 1997, was assigned to Kinetic Concepts Incorporated (K.C.I., San Antonio, TX, USA) and is known as Vacuum Assisted Closure (V.A.C.). This is a foam-based NPWT device, using the patented Therapy Regulated Accurate Care (T.R.A.C.) drainage system to supply the generally recommended suction pressure of -125 mmHg.

The technique of Chariker *et al.* initially described in 1989, is commonly referred to as the Chariker-Jeter technique and entails the use of gauze-based NPWT. Suction is supplied by a perforated drainage tube and the recommended pressures are generally lower than the pressures recommended by K.C.I. (-75 to -80 mmHg). The financial success achieved by the commercialisation of NPWT in the form of the patented V.A.C. device has inspired many other manufacturers to develop

their own form of NPWT. Due to K.C.I. actively protecting its intellectual property, with exorbitant litigation battles still continuing at the present time, practically all other manufacturers have adopted the Chariker-Jeter technique.

Most studies on NPWT have been conducted using the V.A.C. device. The growing number of manufacturers using the Chariker-Jeter technique has resulted in an increasing number of studies on this method in an attempt to clarify which system is the most successful. Some directly compare these two forms of NPWT, while others indirectly "compare" them by testing the outcomes of one type and comparing them to known outcomes of the other. As with many other studies involving NPWT, the outcomes of these comparative studies conflict with one another, despite rigorous scientific methodology and peer-review. Although the laser Doppler study (Chapter 9) had found a possible reason for why many studies had conflicting data, it did not explain why studies comparing the outcomes of NPWT are so divided in their opinion regarding the more successful system (V.A.C. or Chariker-Jeter system). In the current study, an attempt was made to determine whether manufacturer involvement could be related to the outcomes of these scientific studies.

10.2. Hypothesis

The following hypothesis was formulated:

"There is no correlation between the outcome of a study being in favour of the manufacturer and the manufacturer's level of involvement in that study."

10.3. Methods

A literature review was undertaken using Pubmed and Google Scholar, to identify a representative cohort of studies that directly compared these two forms of NPWT. An additional review was undertaken to include studies that did not directly

compare products, but had outcome measures regarding one type of dressing, which assisted our understanding of which of the two systems might be superior. In the latter studies, if the authors did not mention and compare components of the other available system (or their components) they were excluded from the current study as they were not seen as comparative. Clinical outcomes studies, basic research studies and published conference abstracts were included.

10.3.1. Classification techniques

A NPWT system comprises individual components and each of these could be compared or studied separately, as each could affect the success of the system. Therefore, over and above the comparison of the composite V.A.C. and Chariker-Jeter systems, studies comparing the outcomes of the following individual components of a NPWT system were also included: lower suction pressures (Chariker-Jeter technique) vs. the higher ones typically used with V.A.C.; foam (V.A.C.) vs. gauze (Chariker-Jeter technique); single lumen (V.A.C.) vs. perforated drainage tubes (Chariker-Jeter technique).

Therefore, because some studies tested only individual components, while others tested the entire system, instead of referring to V.A.C. or Chariker-Jeter systems, we assigned the products being compared to either *Group A* (any composite V.A.C. system or individual components thereof) or *Group B* (any composite Chariker-Jeter system or individual components thereof) (Table 7). Any study that compared a *Group A* component to its *Group B* counterpart was included in this analysis. Although *Group A* refers to one manufacturer (K.C.I.) and *Group B* to multiple, "*Group B* manufacturers" may often be referred to in the singular form during comparisons, i.e. "*Group B* manufacturer".

Table 7: The two groups, A and B, categorised by specific components known to be part of these systems.

Composite system or components thereof	Group A	Group B
Composite system	V.A.C.	Chariker-Jeter
Interface dressing	Foam (PU or PVA)	Gauze
Suction drainage system	Single lumen (T.R.A.C. pad)	Perforated drainage tube, e.g., Jackson-Pratt drain
Recommended suction pressure	-125 mmHg	-75 to -80 mmHg

Based on their findings and conclusions, studies were then categorised into two groups, namely studies with "outcomes beneficial to *Group A* manufacturers" or those with "outcomes beneficial to *Group B* manufacturers". The current market leaders are K.C.I. (*Group A* manufacturer) with the V.A.C. device, which is still considered the gold-standard in NPWT, albeit more expensive than the *Group B* devices.[29] Therefore, studies that found the *Group B* components to be as good as, or superior to the current gold-standard (*Group A* components), were considered to have "outcomes beneficial to *Group B* manufacturers". If the study concluded that *Group A* components were superior, they were considered to have "outcomes beneficial to *Group A* manufacturers".

Although most studies were quite clear in terms of which system was "as good as" or "superior" to the other, there were some that where less clear. This was found where studies tested multiple components or had various outcome measures and found some components superior for one outcome but not necessarily for others. A study may have also found that only one of the components was superior but that the others were not. But most of these tended to leave the reader with an impression of which system had more overall benefits. For this reason, and to ensure impartial categorisation, all the articles' abstracts and conclusions were given to five surgeons. They were individually asked to record what they would consider to be the "take home" message of each article (in terms of which system is superior).

None of the surgeons had any conflict of interest in either of the two systems. There was no communication between the surgeons during this process. They were blinded to the authors of the study and any manufacturer involvement in the paper. The papers were presented to the surgeons in a randomised manner. This was done by placing the titles of the studies on lots, shuffling these in a closed box and randomly selecting a title prior to the study being presented to the surgeon.

If there was 80% concordance amongst the surgeons' decisions (four out of five), the article was assigned to one of the following categories: "outcomes beneficial to *Group A* manufacturers" or "outcomes beneficial to *Group B* manufacturers". If not, the article was assigned to a third category, namely, *impartial.* Once assigned to one of the three categories, the individual studies were assessed and graded according to the level of manufacturer involvement in the study (Table 8). Only one of the specified criteria was necessary to grade a manufacturers involvement to a particular level.

Table 8: Grading criteria for different levels of manufacturer involvement and associated risk of researcher bias.

Manufacturer involvement level	Criteria	Potential for bias
Level 0	• No manufacturer involvement as per the criteria mentioned below	Negligible
Level 1	• The researchers made exclusive use of a specific manufacturer's suction pump to test products	Low
Level 2	• At least one author is/has been a paid consultant of the manufacturer, or • The manufacturer sponsored all or part of the study, or • Draft manuscript was reviewed by an employee of the manufacturer or anyone with a financial conflict of interest	Medium
Level 3	• Study conducted by manufacturer, or • At least one author is an employee of manufacturer, or • At least one author has a financial conflict of interest (receives royalties, owns shares, etc.)	High

10.3.2. Data analysis

It was planned that an analysis would be done to ascertain whether there was a correlation between the category that a study belonged to ("outcomes beneficial to *Group A manufacturers*" or "outcomes beneficial to *Group B manufacturers*") and the level of manufacturer involvement. However, a formal statistical analysis was deemed not necessary for this aspect of the study after evaluating the outcomes (see results section). The Mann-Whitney U-test was used, however, to compare the levels of involvement of *Group A* and *Group B manufacturers* ($p<0.05$ regarded as significant).

10.4. Results

A total of 24 studies were found to match the inclusion criteria (16 direct and eight indirect comparative studies). The surgeons concurred (more than four out of five in agreement) in 22 of the 24 studies regarding the category to which a study belonged. Two of the 24 studies were therefore added to the *impartial* group. Of the 24 studies, 19 had some form of manufacturer involvement (Levels 1 to 3).

Of the 19 that had some form of manufacturer involvement, 18 had outcomes that were deemed beneficial to the involved manufacturer, while one was considered to have an *impartial* outcome. The correlation between manufacturer involvement, regardless of level, and study outcome was so obvious that statistical analyses were deemed to be unnecessary. In the combined cohort of 24 studies, the outcomes proved beneficial for *Group A* in seven studies and *Group B* in 15, with the remaining two being regarded as having outcomes that do not benefit a particular manufacturer, i.e. *impartial* (Fig. 97).

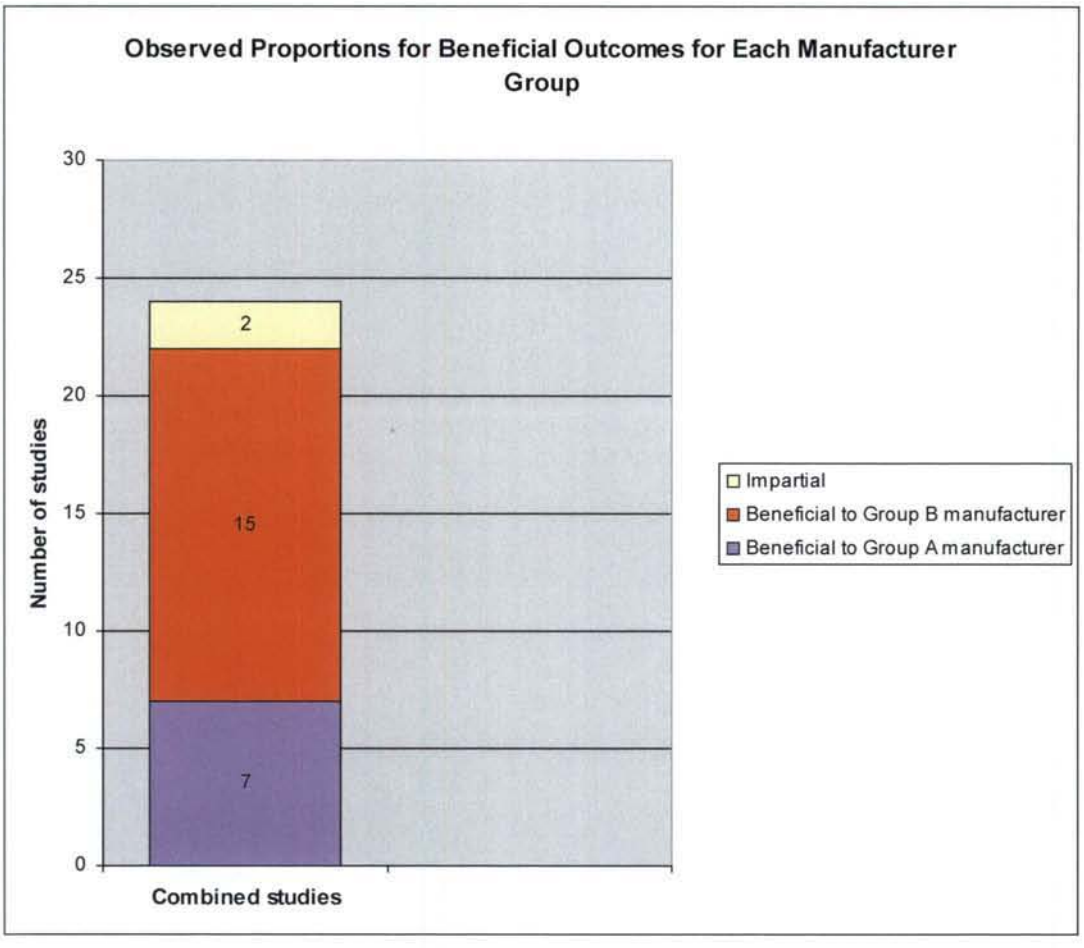

Fig. 98: Graphic representation of the total number of NPWT studies analysed, demonstrating the observed proportion of beneficial outcomes for each manufacturer.

In all the studies (seven) that had outcomes that were beneficial to the *Group A manufacturer*, it was found that the *Group A manufacturer* was involved in the study to a certain level (> Level 0). In fact, in the six direct comparative studies which were beneficial to the *Group A manufacturer*, the involvement was Level 3 in all cases. In the one indirect study, the level of involvement was Level 2 (Table 9).

Table 9: Number of comparative studies with levels of Group A manufacturer involvement (n=7) and study outcomes.

Group A Manufacturer				
Involvement Level	**Direct comparative studies**	**Direct comparative studies with outcomes beneficial to Group A manufacturer**	**Indirect comparative studies**	**Indirect comparative studies with outcomes beneficial to Group A manufacturer**
Level 1	0	0	0	0
Level 2	0	0	1	1
Level 3	6	6	0	0
Totals	6	6	1	1

Of the 15 studies found to have beneficial outcomes for the *Group B manufacturer*, it was found that this manufacturer was involved in 11 of the studies. Of the other four, none was from studies that had *Group A manufacturer* involvement. Instead, they were all from Level 0 studies (no manufacturer involvement). Of the 11 studies that had *Group B manufacturer* involvement with outcomes in favour of this group, seven were direct comparative studies and four were indirect. The *Group B manufacturer* was involved in a total of 12 studies, 11 of which had outcomes that were beneficial to this manufacturer, while one was found to be *impartial* (Table 10).

Table 10: Number of comparative studies with levels of Group B manufacturer involvement (n=12) and study outcomes.

Group B Manufacturer				
Involvement Level	**Direct comparative studies**	**Direct comparative studies with outcomes beneficial to Group B manufacturers**	**Indirect comparative studies**	**Indirect comparative studies with outcomes beneficial to Group B manufacturers**
Level 1	2	2	1	1
Level 2	3	3	2	1 (the other was impartial)
Level 3	2	2	2	2
Totals	7	7	5	4

Of the five Level 0 studies, four were found to be beneficial for the *Group B manufacturer*, while one was deemed *impartial* (Table 11).

Table 11: Number of comparative studies with no manufacturer involvement (Level 0) and study outcomes (n=5).

Level 0 studies (no manufacturer involvement)		
Outcomes	**Direct comparative studies**	**Indirect comparative studies**
Beneficial to Group A manufacturer	0	0
Beneficial to Group B manufacturer	2	2
Impartial	1	0
Totals	3	2

In the combined cohort (direct and indirect comparative studies), there was a significantly higher level of involvement for the *Group A manufacturer* than there was for *Group B manufacturer* (p=0.03), with levels of involvement shown in Table 12. The median level for *Group A* was 3 and *Group B* was 2.

Table 12: Levels of manufacturer involvement of direct and indirect comparative studies (with involved manufacturer group appearing in brackets).

Manufacturer involvement	Direct comparative studies	Indirect comparative studies	Totals
Level 0	3	2	5
Level 1	2 (both Group B)	1 (Group B)	3
Level 2	3 (all Group B)	3 (2 Group B, 1 Group A)	6
Level 3	8 (6 Group A, 2 Group B)	2 (both Group B)	10
Totals	16 (6 Group A, 7 Group B, 3 Level 0)	8 (1 Group A, 5 Group B, 2 Level 0)	24

10.5. Discussion

This study found compelling evidence that the outcomes of research studies in NPWT appear to be influenced by the involvement of manufacturers. Except for one study, all the studies in which there was manufacturer involvement had outcomes that were beneficial to the manufacturer involved in the study, irrespective of the level of involvement.

When analysing each group with manufacturer involvement, if the proportions of the outcomes were roughly similar to those of the total number of studies (Fig. 98), then it could be assumed that there is little influence by the manufacturer on the study outcome. However, they were found to be disproportionately in favour of the involved manufacturer to a considerable degree (Fig. 99), implying that any form of manufacturer involvement appears to affect the outcome of the study in favour of that manufacturer.

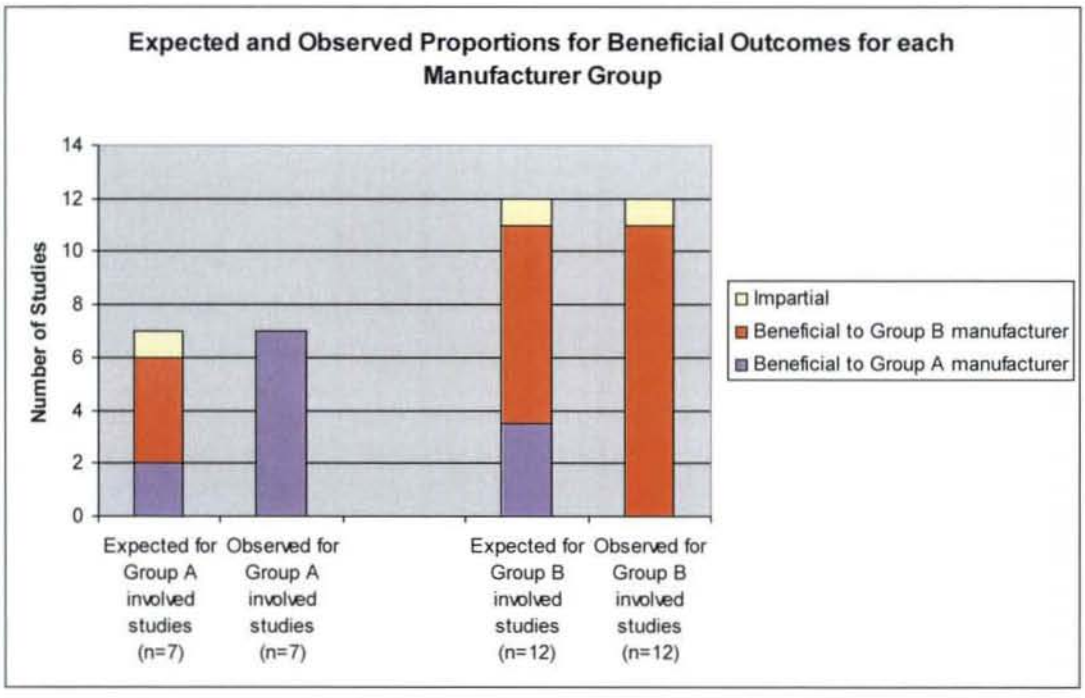

Fig. 99: Expected and observed proportions of study outcomes for studies with Group A or B manufacturer involvement.

This does not necessarily imply any devious behaviour on the part of the authors of those studies, as they often had no conflict of interest to declare. Instead, it may simply reflect human nature and our subconscious desire to want positive outcomes for those that assist us in whatever we undertake. Researchers may, therefore, be unintentionally or subconsciously biased in these studies.

Unfortunately, as a result of this influence our understanding of the biomechanics of NPWT, such as the mechanical effects of gauze and foam and the ideal pressure to use, may have been clouded by such studies. Yet, it is these studies that have the power to influence clinical guidelines worldwide or the course of further research.

10.6. Conclusion

Sufficient evidence has been found to suggest that manufacturer involvement in studies correlates with the outcomes being beneficial to the involved manufacturer, regardless of the level of involvement. The hypothesis is therefore rejected.

Studies that have some form of manufacturer involvement need to be scrutinised very critically in our peer-review system. Ideally, there should be no manufacturer involvement in any study comparing competing manufacturer's products. When this is not possible, both manufacturers should be equally involved in the comparative study.

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11 Conclusion and Further Work

Outline

11.1. Introduction

This research project was born out of a question raised as a result of an adverse clinical outcome following the application of NPWT. The core of this research was to determine whether NPWT increases or decreases tissue pressure and perfusion.

During preliminary experiments it was also questioned whether the mechanics of different dressing configurations would result in different findings. It was, therefore, decided to investigate all three configurations that a NPWT dressing may assume, namely, circumferential, non-circumferential or a NPWT dressing in a cavity.

Tissue pressure experiments in the above-mentioned dressing configurations were undertaken on inanimate substances and on human wounds. The effects of suction pressure, length of time of application, and dressing configuration were evaluated. The findings of the tissue pressure experiments prompted the investigation of tissue perfusion research in circumferential and non-circumferential dressing configurations.

The contention that perfusion may be reduced adjacent to the dressing but increased about 1-3 cm away[1-5] prompted a thermographic evaluation of the tissue around a NPWT dressing.

The findings of the perfusion studies that have been presented herein conflict with those of many others.[1-17] Most of these studies were conducted with a laser Doppler and, of these, some reported findings that were incongruous with the others. As the laser Doppler was the common denominator, potential flaws in the devices measuring technique were looked for and identified. Experiments were then conducted to test whether these theoretical flaws existed in reality.

It was also noticed that when there was some form of manufacturer involvement in studies that compared the two most commonly used NPWT systems (V.A.C. or Chariker-Jeter), the outcomes appeared to be beneficial for the involved manufacturer. A study was undertaken to see whether there was indeed a correlation between manufacturer involvement and study outcome.

11.2. Study results

Results of these studies are summarised below:

1. All three configurations of NPWT tested resulted in an immediate increase in mean tissue pressure (in *in vitro* and *in vivo* studies), for all suction pressures.
2. An *in vitro* test demonstrated that this increased pressure dissipated rapidly as the distance from the dressing increased.
3. The increased tissue pressure was directly proportional to the amount of suction applied.
4. The mean increase in tissue pressures in the circumferential dressing group was significantly greater than those of the non-circumferential group or cavity group, although the difference between the latter two groups was not statistically significant.
5. The increased tissue pressure decreased significantly in both the circumferential and non-circumferential NPWT dressings over a 48-hour period (the cavity dressing configuration was not tested).
6. The mean reduction of the increased tissue pressure was significantly more in the non-circumferential dressing configuration than in the circumferential group.
7. Perfusion was significantly decreased in both circumferential and non-circumferential NPWT dressings.

8. The mean reduction in perfusion was greater at gauge pressures of -400 mmHg than at -125 mmHg, in both circumferential and non-circumferential NPWT dressings, although this finding was statistically significant only in the circumferential group.
9. There was no thermographic evidence in support of a zone of increased perfusion surrounding the zone of reduced perfusion adjacent to the dressing, at any of the gauge pressures tested (-75 mmHg, -125 mmHg or -400 mmHg).
10. There was no correlation between suction pressure and change in skin temperature (perfusion) at distances between 1 and 3 cm from the dressing.
11. The laser Doppler was shown to be a flawed modality to measure perfusion in NPWT research.
12. Involvement of a manufacturer in a study on NPWT is highly likely to influence the outcomes of that study in favour of that specific manufacturer.

11.3. Discussion

11.3.1. NPWT and tissue pressure

In keeping with the findings of Maier *et al.*[18] and Willy *et al.*,[19] NPWT was shown to increase tissue pressure for increasing suction pressure, contrary to common perception. Although not tested in human wounds, *in vitro* studies demonstrated that this pressure rapidly dissipates as the distance from the dressing increases. This distance-related dissipation of tissue pressure is likely to vary depending on the type and consistency of the tissue, type of interface dressing, size and configuration of interface dressing and dimensions of the wound. This was not formally shown in this research but was suggested by the findings of the preliminary studies.

The increased tissue pressure also demonstrated a time-related dissipation, which was more significant in the non-circumferential group. Again, this is likely to be influenced by the factors mentioned in the preceding paragraph. The reasons for this time-related dissipation of increased tissue pressure are not known. It may be due to a reduction in oedema with an associated reduction in interstitial fluid pressure. Alternatively, the reduction in oedema is likely to result in a change in the consistency of tissues, and thereby the tissues' pressure-transducing capabilities. A third possibility exists, namely, that the tissues around the pressure transducer gradually conform to the shape of the transducer, thereby resulting in less pressure being applied to the sensing window. Indeed, it may be possible that a combination of these factors might be responsible for the observed findings.

The circumferential NPWT dressings resulted in a greater increase in tissue pressure then the other configurations. The reduction of this increased tissue pressure was also less pronounced in this group. Again, the reasons for this finding can only be speculated upon. It may be that the circumferential nature of the dressing results in an additional constricting effect, a quality which the others dressing configurations do not possess. However, it was noted in the preliminary research ("Vacolitre" Experiments) that changing the foam application from a "wrap-around" to a "sandwich" technique did not result in a lesser increase in substance pressure. In fact, this increased the pressure even further.

As double the amount of foam was used for the sandwich technique (two slabs, instead of one), this raised the question of whether the quantity of foam used may play a role in how much the tissue pressure increases. Although, these were only informal studies, the "Vacolitre" experiments showed this to be true, when four slabs were used instead to two. This was also shown in another preliminary experiment, when testing the pressure effects of non-circumferential NPWT on a rigid surface ("Table" experiments). It was demonstrated that doubling the volume of the NPWT foam increased the underlying substance pressure more than if only

the thickness were doubled (with the volume kept constant). As a greater amount of interface dressing (volume) is typically required to create a circumferential NPWT dressing than one which is not circumferential, this may be a reason for the greater amount of increased tissue pressure observed in the circumferential NPWT group.

The scientific explanation for the increased tissue pressure generated by all configurations of NPWT dressings is still the subject of on-going research. Understanding the physics of this NPWT will allow us to predict how the pressure within a particular tissue will change in response to a particular NPWT dressing. Additionally, this knowledge may advance our understanding of the mechanism of action of NPWT and help design NPWT iterations that result in better outcomes than current versions.

11.3.2. NPWT and perfusion

In contrast to all studies undertaken in the past that assessed the immediate changes in perfusion during NPWT,[1-17] the studies presented in this thesis did not demonstrate any evidence of increased perfusion. In fact, the studies showed significant reductions in perfusion, regardless of dressing configuration. This was true even for the frequently quoted suction pressure of -125 mmHg, which has been accepted by most in the field to result in a fourfold increase in perfusion, following the seminal work of Morykwas *et al.*[6]

The studies of the Lund researchers are partly in keeping with the work of this thesis, in that they too found reduced perfusion adjacent to the dressing.[1-5] However, the Lund researchers demonstrated an increase in perfusion between 1 and 3 cm from the wound, concluding that a zone of hyperperfusion exists surrounding the zone of hypoperfusion. This finding, however, was difficult to explain given the fact that the studies presented in Chapter 4 and 5 showed only increases in tissue pressure. Additionally, the tissue pressure was never shown

to be hypobaric at any given distance from the dressing, and therefore was incongruous with the concept of a zone of increased perfusion. The thermography study (Chapter 8) did not find any evidence that a zone of hyperperfusion exists between the distances of 1 and 3 cm from the dressing.

11.3.3. Flaws in the methodology of prior research

The evidence presented herein appears to follow a logical progression, in that it has been shown unequivocally that NPWT increases tissue pressure and, as a consequence, the increased tissue pressure reduces perfusion. Despite the fact that this makes scientific and physiological sense, it conflicts with our prior understanding of how the hypobaric pressure of a NPWT dressing affects tissue pressure and perfusion. It was commonly believed that the hypobaric pressure of the NPWT created a pressure gradient, which resulted in a surge of blood to the wound.[11, 12, 20-22]

The studies presented herein demonstrated that the opposite happens. Although a pressure gradient is created, the directionality of the gradient is the opposite of what was commonly assumed. Rather than a zone of decreased pressure existing around the NPWT dressing, which results in blood being drawn to the wound, a zone of increased pressure exists, resulting in blood being forced away from the wound. The fact that this conflicts with prior research is explained by potential flaws of prior research.

Although potential flaws of studies have been highlighted in Chapter 1, the flaw that was most concerning related to the conflicting perfusion findings. Not only did studies often conflict with one another's findings, but they were in contradistinction to the studies presented in this thesis. The majority of perfusion studies have been conducted with the laser Doppler. The laser Doppler study (Chapter 9) demonstrated that this device is inherently flawed in its technique of measuring perfusion when used in conjunction with NPWT.

The laser Doppler was found to be inconsistent, as it was seen to record an increase in perfusion in some regions during NPWT, yet a reduction in other regions. This was also found to be the case when applying manual pressure to the dressing without suction. Furthermore, the fact that the study demonstrated that it was often possible to create an apparent increase in perfusion by merely applying manual pressure to the foam (in the form of a weight), demonstrates the design flaw of this modality in NPWT research. Applying manual pressure to any living tissue should always be expected to reduce perfusion.

These inconsistent findings and the fact that it is possible to demonstrate an apparent increase in perfusion with manual pressure, provide adequate reason for the contradictory findings of previous studies using this modality. In essence, this finding invalidates all prior studies on NPWT and perfusion that utilised laser Doppler. This does not imply that the researchers' study design was inadequate or of a low standard, but merely that they were misled by this device.

11.3.4. The manufacturers' influence on NPWT study outcomes

NPWT research appears to be characterised by conflicting findings but fortunately the laser Doppler study appears to have partly answered why this is so. Another potential confounding factor in NPWT research appears to be whether a manufacturer has some form of involvement in the study. Chapter 10 demonstrated that nearly every study in which a particular manufacturer had some level of involvement resulted in outcomes that were beneficial to that manufacturer's NPWT system. Our understanding of the biomechanics of gauze and foam and the ideal pressure to use in NPWT may have been clouded by such studies. Chapter 10 demonstrates that if we want to compare NPWT systems of a different nature, there needs to be absolutely no manufacturer involvement in that study or both manufacturers should be equally involved at the very least.

The study conducted in Chapter 10 does not imply any devious behaviour on the

part of the authors of those studies, as they often had no conflict of interest to declare. Instead, it may simply reflect human nature and our desire to want positive outcomes for those that assist us in whatever we undertake. Researchers may, therefore, have been unintentionally or subconsciously biased in these studies.

11.4. Conclusions

There was no evidence found to support the common perception that NPWT reduces tissue pressure and increases perfusion. In fact, sufficient evidence was found to prove that the opposite occurs, namely, it increases tissue pressure and reduces perfusion.

It became apparent that the reason researchers were misled into thinking that perfusion was increased, was due to an inherent flaw in the measuring technique of the laser Doppler device. This modality was proven to be inappropriate to use during NPWT research. Manufacturer involvement in studies appears to be an additional confounding factor that should be avoided if possible.

11.5. Novel theories on the mechanism of action of NPWT

The resultant paradigm shift in our understanding of the biomechanics of NPWT and its effects on tissue pressure and perfusion has resulted in novel theories regarding the mechanism of action of these dressings:

1. The compression of tissue by the NPWT dressing will decrease perfusion beneath the foam. These sheer forces and concurrent hypoxia are recognised stimuli for angioneogenesis, a key component of granulation tissue formation.[23-25]
2. Tissue hypoxia results in release of nitric oxide and local vasodilatation.[25] This aspect, however, would only be beneficial once the suction is removed

or during the "suction off" periods of intermittent NPWT. The perfusion during this "off" period is increased due to the reactive hyperaemia,[2, 7] which is likely to follow. This may explain why intermittent NPWT has been found to be more advantageous than continuous.[6]

3. Physiological interstitial fluid hydrostatic pressure is usually hypobaric (about -1 mmHg).[26-30] It has been demonstrated that with injury, this hypobaric pressure can rapidly decrease even further (up to -30 mmHg).[29, 30] This, together with increased permeability of vessels due to inflammation, greatly contributes to the formation of oedema. The increased tissue pressure generated by NPWT is likely to reduce, if not reverse, this process.

4. Compression of a vessel through which fluid is flowing results in increased velocity of the fluid (known in physics as the Principle of Continuity). When fluid velocity is increased in a vessel, this fluid's hydrostatic pressure decreases (Bernoulli's principle). This reduced intravascular pressure, in combination with the increased tissue pressure, would result in less efflux of plasma from the vessel, resulting in decreased oedema. This theory, however, may not hold true in NPWT as the increase in tissue pressure may nullify the reduced pressure within the vessels and further research is warranted to test this theory.

5. The increased blood velocity is more likely to draw extracellular fluid into the vessel, on the basis of the Venturi principle (Fig. 99).

6. In addition to the oedema reduction of the last two mechanisms, the compressive forces of NPWT may physically force oedema away from the injured tissues in a similar manner to an anti-oedema garment. This will ultimately result in less interstitial hydrostatic pressure, less compression of the vessels and also improved oxygen and nutrient diffusion to the cells.

7. Splinting of the wound, which is achieved by this positive pressure, also aids in wound healing.[31, 32]

The above theories predominantly consider perfusion and oedema reduction, but other factors also contribute. The theory on microdeformation/microstrain at the wound interface[33] causing effects similar to tissue expansion, with release of growth factors, offers another mechanism. Although the net pressure inside the tissue is positive, the pressure on the portion of tissue cells directly beneath a pore of the foam may in fact still be negative, resulting in micro-expansion on those particular cells. This could explain why granulation tissue occurs much faster beneath the black polyurethane foam, which has larger pores than the white polyvinyl alcohol foam. This theory has found support in recent studies.[34,35] Similar effects also occur at a macroscopic level (macrostrain), when wound edges are pulled closer to one another when the interface dressing volume reduces in size.

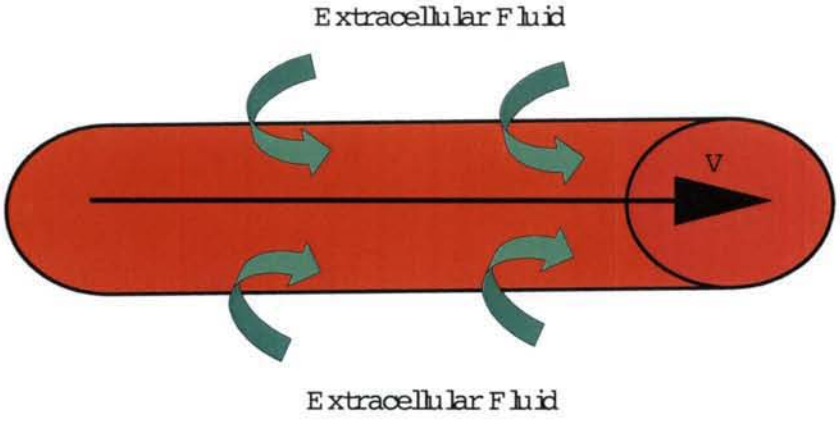

Fig. 100: As blood velocity increases, an increasing amount of extracellular fluid is drawn into the vessel, based on the Venturi Principle.

11.6. Further work

With the development of novel theories comes the creation of new avenues of research. These will primarily tend to explore the mechanism of action of NPWT and also the potential for adverse outcomes.

Further work needs to be done to clarify why NPWT increases tissue pressure

and what factors influence this. This will afford us more control when we need to manipulate tissue pressure to improve wound healing or to prevent adverse outcomes, such as tissue necrosis.

More importantly, work needs to be done to determine why wounds respond favourably to the increased tissue pressure and the associated reduced perfusion. This will help to explain the mechanism of action of NPWT to a large extent. Armed with this knowledge, more advanced NPWT devices can be designed, which may provide even better outcomes.

This research has highlighted that the possible deleterious effects of NPWT are related to their potential to reduce perfusion. Therefore, further work needs to be done to understand the degree of hypoxia that NPWT dressings can elicit for given suction pressures, types of dressing interfaces, dressing configurations and types of tissues. In particular, the proposal that it improves myocardial perfusion[8-10, 14, 17] needs to be cautiously investigated prior to any human studies being undertaken. The research presented in this thesis does not support such a proposal.

Despite the fact that perfusion is reduced, one needs to consider whether this reduction is of such a magnitude that it outweighs the advantages of the increased tissue pressure. The increased tissue pressure and the associated reduction in oedema may be more beneficial in improving the oxygenation of tissues than an increase in perfusion. In reality a balance between optimal tissue pressure and vascular inflow is likely to be the ideal scenario. Further research needs to be done, similar to the work of Morykwas *et al.*,[6] where skin flaps are raised on pigs. As in Morykwas' study, the proportions of these flaps should be such that part of the flap will undergo necrosis. The effects of different types of NPWT dressings and suction pressures should then be tested to evaluate whether oedema reduction or perfusion reduction appear to be more important in tissue survival.

11.7. Summary

In summary, this research demonstrates that the immediate action of NPWT is to generate an increase in tissue pressure, and that this results in a reduction in perfusion. This appears to be more exaggerated in circumferential NPWT dressings and higher suction pressures. The increased tissue pressure reduces with time.

The evidence does not support the existence of a zone of increased perfusion around the dressing. Furthermore, sufficient evidence was found to invalidate the laser Doppler as a modality to measure perfusion in NPWT research. Evidence was also found that our knowledge on NPWT may have been clouded by manufacturer involvement in research studies.

The paradigm shift represented in this research has explained many conflicting findings but has generated an equal number of new questions and further research is warranted.

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Appendix A.
Experiment data: NPWT and tissue pressure - In vitro *experiments*

A1. Circumferential NPWT

Table A.1: Substance pressure recorded in a sausage exposed to increasing levels of suction from a circumferential NPWT dressing.

Suction pressure (mmHg)	Tissue Pressure (mmHg)								
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Mean	SD	Min	Max
0	0	0	0	0	0	0	0.00	0	0
-100	12	10	12	12	16	12.4	2.19	10	16
-200	22	24	25	29	26	25.2	2.59	22	29
-300	33	35	28	40	34	34	4.30	28	40
-400	38	40	39	50	46	42.6	5.18	38	50
-500	42	52	48	47	51	48	3.94	42	52

A2. Non-circumferential NPWT

Table A.2: Substance pressure recorded in Plasticine exposed to increasing levels of suction from a non-circumferential NPWT dressing.

Suction pressure (mmHg)	Tissue Pressure (mmHg)								
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Mean	SD	Min	Max
0	0	0	0	0	0	0	0.00	0	0
-100	10	12	11	12	13	11.6	1.14	10	13
-200	29	28	28	28	28	28.2	0.45	28	29
-300	38	40	40	41	42	40.2	1.48	38	42
-400	46	47	49	48	50	48	1.58	46	50
-500	50	51	51	51	56	51.8	2.39	50	56

A3. NPWT placed in a cavity

Table A.3: Substance pressure recorded at a depth of 1 cm from the base of a cavity in processed meat, exposed to increasing levels of suction from a NPWT dressing.

Suction pressure (mmHg)	Tissue Pressure (mmHg)								
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Mean	SD	Min	Max
0	0	0	0	0	0	0	0.00	0	0
-100	4	5	5	6	9	5.8	1.92	4	9
-200	9	17	19	21	23	17.8	5.40	9	23
-300	19	26	26	29	33	26.6	5.13	19	33
-400	21	29	33	36	37	31.2	6.50	21	37
-500	25	34	38	39	41	35.4	6.35	25	41

Table A.4: Substance pressure recorded at a depth of 1 cm from the wall of a cavity in processed meat, exposed to increasing levels of suction from a NPWT dressing.

Suction pressure (mmHg)	Tissue Pressure (mmHg)								
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Mean	SD	Min	Max
0	0	0	0	0	0	0	0.00	0	0
-100	6	8	9	10	12	9	2.24	6	12
-200	12	13	14	15	16	14	1.58	12	16
-300	16	16	19	19	20	18	1.87	16	20
-400	18	22	22	23	25	22	2.55	18	25
-500	24	24	26	27	29	26	2.12	24	29

Table A.5: Substance pressure recorded at a depth of 2 cm from the wall of a cavity in processed meat, exposed to increasing levels of suction from a NPWT dressing.

Suction pressure (mmHg)	Tissue Pressure (mmHg)								
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Mean	SD	Min	Max
0	0	0	0	0	0	0	0.00	0	0
-100	1	2	2	3	2	2	0.71	1	3
-200	3	4	6	6	6	5	1.41	3	6
-300	4	6	8	8	9	7	2.00	4	9
-400	4	6	8	8	9	7	2.00	4	9
-500	6	6	9	9	10	8	1.87	6	10

Table A.6: Substance pressure recorded at a depth of 3 cm from the wall of a cavity in processed meat, exposed to increasing levels of suction from a NPWT dressing.

Suction pressure (mmHg)	Tissue Pressure (mmHg)								
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Mean	SD	Min	Max
0	0	0	0	0	0	0	0.00	0	0
-100	1	1	1	1	1	1	0.00	1	1
-200	1	1	1	1	1	1	0.00	1	1
-300	1	1	1	1	1	1	0.00	1	1
-400	2	2	2	2	2	2	0.00	2	2
-500	2	2	2	2	2	2	0.00	2	2

Appendix B.
Experiment data: NPWT and tissue pressure - In vivo experiments

B1. Circumferential NPWT

B1.1. Tissue pressure changes at increasing levels of suction from circumferential NPWT dressings.

Table B.1: Tissue pressure changes recorded over the proximal phalanx (P1), post palmar graft, in the finger of a hand exposed to increasing levels of suction from a circumferential NPWT dressing.

Suction pressure (mmHg)	Tissue Pressure (mmHg)								
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Mean	SD	Min	Max
0	0	0	0	0	0	0	0.00	0	0
-75	34	30	23	20	19	25.2	6.53	19	34
-150	55	43	38	35	31	40.4	9.26	31	55
-225	69	58	51	46	43	53.4	10.41	43	69
-300	83	68	61	56	54	64.4	11.72	54	83
-375	88	70	63	62	58	68.2	11.88	58	88
-450	90	71	63	64	60	69.6	12.10	60	90

Table B.2: Tissue pressure changes recorded beneath flap of a hand exposed to increasing levels of suction from a circumferential NPWT dressing.

Suction pressure (mmHg)	Tissue Pressure (mmHg)								
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Mean	SD	Min	Max
0	0		0	0	0	0	0.00	0	0
-75	12	9	10	11	10	10.4	1.14	9	12
-150	18	19	17	18	17	17.8	0.84	17	19
-225	24	25	24	25	23	24.2	0.84	23	25
-300	32	32	31	32	29	31.2	1.30	29	32
-375	38	35	35	36	35	35.8	1.30	35	38
-450	41	40	40	40	39	40	0.71	39	41

Table B.3: Tissue pressure changes recorded over the dorsum of the middle phalanx (P2) of a finger of a hand exposed to increasing levels of suction from a circumferential NPWT dressing.

Suction pressure (mmHg)	Tissue Pressure (mmHg)								
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Mean	SD	Min	Max
0	0	0	0	0	0	0	0.00	0	0
-75	12	15	12	14	15	13.6	1.52	12	15
-150	18	18	16	18	17	17.4	0.89	16	18
-225	24	22	20	23	22	22.2	1.48	20	24
-300	26	25	25	25	26	25.4	0.55	25	26
-375	29	27	27	27	28	27.6	0.89	27	29
-450	30	28	29	29	28	28.8	0.84	28	30

Table B.4: Tissue pressure changes recorded in the second webspace of a hand exposed to increasing levels of suction from a circumferential NPWT dressing

Suction pressure (mmHg)	Tissue Pressure (mmHg)								
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Mean	SD	Min	Max
0	0	0	0	0	0	0	0.00	0	0
-75	9	7	8	8	9	8.2	0.84	7	9
-150	13	13	12	12	12	12.4	0.55	12	13
-225	17	16	16	16	15	16	0.71	15	17
-300	21	19	20	19	20	19.8	0.84	19	21
-375	23	23	23	23	23	23	0.00	23	23
-450	25	26	25	25	26	25.4	0.55	25	26

Table B.5: Tissue pressure changes recorded over the dorsum of the third metacarpophalangeal joint (MCPJ) of a hand exposed to increasing levels of suction from a circumferential NPWT dressing.

Suction pressure (mmHg)	Tissue Pressure (mmHg)								
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Mean	SD	Min	Max
0	0	0	0	0	0	0	0.00	0	0
-75	3	3	3	3	3	3	0.00	3	3
-150	10	9	9	9	8	9	0.71	8	10
-225	13	13	13	14	13	13.2	0.45	13	14
-300	17	16	16	16	16	16.2	0.45	16	17
-375	20	19	19	20	19	19.4	0.55	19	20
-450	23	23	23	23	23	23	0.00	23	23

B1.2. Tissue pressure over a 48-hour period at a constant level of suction from circumferential NPWT dressings.

Table B.6: Tissue pressure recorded over a 48-hour period over the middle phalanx (P2) dorsum in the finger of a hand exposed to circumferential NPWT at a constant suction pressure of -125 mmHg. Measurements were taken hourly and the means of 4-hourly intervals were obtained.

Time period	Mean tissue pressure (mmHg) of 4-hourly intervals
0 to 4	15
4 to 8	15.2
8 to 12	14.8
12 to 16	14.4
16 to 20	15.2
20 to 24	14
24 to 28	13.4
28 to 32	13.2
32 to 36	13.2
36 to 40	11.6
40 to 44	12.6
44 to 48	12

Table B.7: Tissue pressure recorded over a 48-hour period in the second webspace of a hand exposed to circumferential NPWT at a constant suction pressure of -125 mmHg. Measurements were taken hourly and the means of 4-hourly intervals were obtained.

Time period	Mean tissue pressure (mmHg) of 4-hourly intervals
0 to 4	9
4 to 8	5.6
8 to 12	3.5
12 to 16	1.5
16 to 20	3
20 to 24	1.5
24 to 28	1.25
28 to 32	4
32 to 36	3.75
36 to 40	1.75
40 to 44	2.25
44 to 48	2

Table B.8: Tissue pressure recorded over a 48-hour period over the third metacarpophalangeal joint (MCPJ) of a hand exposed to circumferential NPWT at a constant suction pressure of -125 mmHg. Measurements were taken hourly and the means of 4-hourly intervals were obtained.

Time period	Mean tissue pressure (mmHg) of 4-hourly intervals
0 to 4	9
4 to 8	8.25
8 to 12	6.75
12 to 16	7
16 to 20	7.5
20 to 24	9.75
24 to 28	9.75
28 to 32	12.75
32 to 36	10
36 to 40	7.5
40 to 44	7.25
44 to 48	9.75

Table B.9: Tissue pressure recorded over a 48-hour period beneath a flap on a hand exposed to circumferential NPWT at a constant suction pressure of -125 mmHg. Measurements were taken hourly and the means of 4-hourly intervals were obtained.

Time period	Mean tissue pressure (mmHg) of 4-hourly intervals
0 to 4	16.5
4 to 8	20.25
8 to 12	14.25
12 to 16	5
16 to 20	2.5
20 to 24	-0.5
24 to 28	0.5
28 to 32	-0.25
32 to 36	0.5
36 to 40	3.5
40 to 44	0
44 to 48	-0.325

Table B.10: Tissue pressure recorded over a 48-hour period over the proximal phalanx (P1), post palmar graft, in the finger of a hand exposed to circumferential NPWT at a constant suction pressure of -125 mmHg. Measurements were taken hourly and the means of 4-hourly intervals were obtained.

Time period	Mean tissue pressure (mmHg) of 4-hourly intervals
0 to 4	10
4 to 8	11.25
8 to 12	14.25
12 to 16	15.25
16 to 20	19
20 to 24	18.75
24 to 28	17.5
28 to 32	10
32 to 36	12
36 to 40	11.5
40 to 44	12.5
44 to 48	13.5

B2. Non-circumferential NPWT

B2.1. Tissue pressure changes at increasing levels of suction from non-circumferential NPWT dressings.

Table B.11: Tissue pressure changes recorded over a forearm exposed to increasing levels of suction from a non-circumferential NPWT dressing.

Suction pressure (mmHg)	Tissue Pressure (mmHg)								
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Mean	SD	Min	Max
0	0	0	0	0	0	0	0.00	0	0
-75	7	8	8	9	7	7.8	0.84	7	9
-150	9	9	10	9	8	9	0.71	8	10
-225	11	10	12	11	11	11	0.71	10	12
-300	13	12	13	13	12	12.6	0.55	12	13
-375	15	14	15	15	14	14.6	0.55	14	15
-450	16	15	16	15	16	15.6	0.55	15	16

Table B.12: Tissue pressure changes recorded over a heel exposed to increasing levels of suction from a non-circumferential NPWT dressing.

Suction pressure (mmHg)	Tissue Pressure (mmHg)								
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Mean	SD	Min	Max
0	0	0	0	0	0	0	0.00	0	0
-75	5	4	5	5	5	4.8	0.45	4	5
-150	8	7	8	8	8	7.8	0.45	7	8
-225	9	10	9	10	9	9.4	0.55	9	10
-300	10	11	10	11	11	10.6	0.55	10	11
-375	11	12	12	12	12	11.8	0.45	11	12
-450	13	13	13	13	13	13	0.00	13	13

Table B.13: Tissue pressure changes recorded in a thin thigh exposed to increasing levels of suction from a non-circumferential NPWT dressing.

Suction pressure (mmHg)	Tissue Pressure (mmHg)								
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Mean	SD	Min	Max
0	0	0	0	0	0	0	0.00	0	0
-75	1	1	3	3	4	2.4	1.34	1	4
-150	3	4	5	6	6	4.8	1.30	3	6
-225	5	5	6	7	6	5.8	0.84	5	7
-300	6	6	6	7	7	6.4	0.55	6	7
-375	7	7	6	8	8	7.2	0.84	6	8
-450	8	8	7	9	9	8.2	0.84	7	9

Table B.14: Tissue pressure changes recorded over a scalp exposed to increasing levels of suction from a non-circumferential NPWT dressing.

Suction pressure (mmHg)	Tissue Pressure (mmHg)								
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Mean	SD	Min	Max
0	0	0	0	0	0	0	0.00	0	0
-75	2	3	3	4	3	3	0.71	2	4
-150	3	4	4	5	4	4	0.71	3	5
-225	3	5	5	5	5	4.6	0.89	3	5
-300	4	6	6	5	6	5.4	0.89	4	6
-375	4	6	6	6	7	5.8	1.10	4	7
-450	5	7	7	7	7	6.6	0.89	5	7

Table B.15: Tissue pressure changes recorded in a fat thigh exposed to increasing levels of suction from a non-circumferential NPWT dressing.

Suction pressure (mmHg)	Tissue Pressure (mmHg)								
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Mean	SD	Min	Max
0	0	0	0	0	0	0	0.00	0	0
-75	0	0	0	1	1	0.4	0.55	0	1
-150	1	1	1	2	1	1.2	0.45	1	2
-225	1	2	1	2	2	1.6	0.55	1	2
-300	2	2	2	3	2	2.2	0.45	2	3
-375	2	2	2	3	3	2.4	0.55	2	3
-450	3	3	2	3	3	2.8	0.45	2	3

B2.2. Tissue pressures over a 48-hour period at a constant level of suction from non-circumferential NPWT dressings.

Table B.16: Tissue pressure recorded over a 48-hour period in a forearm exposed to non-circumferential NPWT at a constant suction pressure of -125 mmHg. Measurements were taken hourly and the means of 4-hourly intervals were obtained.

Time period	Mean tissue pressure (mmHg) of 4-hourly intervals
0 to 4	10
4 to 8	9.5
8 to 12	6
12 to 16	6.25
16 to 20	4.25
20 to 24	3
24 to 28	1.75
28 to 32	2
32 to 36	1.5
36 to 40	-0.5
40 to 44	-0.75
44 to 48	-2

Table B.17: Tissue pressure recorded over a 48-hour period in a scalp exposed to non-circumferential NPWT at a constant suction pressure of -125 mmHg. Measurements were taken hourly and the means of 4-hourly intervals were obtained.

Time period	Mean tissue pressure (mmHg) of 4-hourly intervals
0 to 4	6
4 to 8	10.25
8 to 12	12.75
12 to 16	13.25
16 to 20	9.5
20 to 24	10.75
24 to 28	10.5
28 to 32	5.75
32 to 36	3.25
36 to 40	0.75
40 to 44	-0.25
44 to 48	-1

Table B.18: Tissue pressure recorded over a 48-hour period in a thin thigh exposed to non-circumferential NPWT at a constant suction pressure of -125 mmHg. Measurements were taken hourly and the means of 4-hourly intervals were obtained.

Time period	Mean tissue pressure (mmHg) of 4-hourly intervals
0 to 4	5.75
4 to 8	4.5
8 to 12	5
12 to 16	4.25
16 to 20	4.75
20 to 24	4.5
24 to 28	5
28 to 32	4
32 to 36	4.25
36 to 40	4.5
40 to 44	5
44 to 48	5.25

Table B.19: Tissue pressure recorded over a 48-hour period in a heel wound exposed to non-circumferential NPWT at a constant suction pressure of -125 mmHg. Measurements were taken hourly and the means of 4-hourly intervals were obtained.

Time period	Mean tissue pressure (mmHg) of 4-hourly intervals
0 to 4	3.5
4 to 8	7.25
8 to 12	4.75
12 to 16	4.75
16 to 20	1.5
20 to 24	2
24 to 28	3.25
28 to 32	1.25
32 to 36	1.75
36 to 40	2.5
40 to 44	2
44 to 48	2.75

Table B.20: Tissue pressure recorded over a 48-hour period in a fat thigh exposed to non-circumferential NPWT at a constant suction pressure of -125 mmHg. Measurements were taken hourly and the means of 4-hourly intervals were obtained.

Time period	Mean tissue pressure (mmHg) of 4-hourly intervals
0 to 4	1
4 to 8	1
8 to 12	1.5
12 to 16	1
16 to 20	2
20 to 24	1.5
24 to 28	1.25
28 to 32	1
32 to 36	2
36 to 40	2
40 to 44	1.25
44 to 48	0.25

B3. NPWT in cavities

B3.1. Tissue pressure changes in cavity wounds exposed to increasing levels of suction from NPWT dressings.

Table B.21: Tissue pressure changes recorded at a depth of 1 cm from the wall of an ischial bedsore cavity exposed to increasing levels of suction from a NPWT dressing.

Suction pressure (mmHg)	Tissue Pressure (mmHg)								
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Mean	SD	Min	Max
0	0	0	0	0	0	0	0.00	0	0
-75	1	2	2	1	1	1.4	0.55	1	2
-150	2	2	2	1	2	1.8	0.45	1	2
-225	2	2	2	2	2	2	0.00	2	2
-300	3	3	2	3	3	2.8	0.45	2	3
-375	4	3	3	4	4	3.6	0.55	3	4
-450	4	4	4	4	4	4	0.00	4	4

Table B.22: Tissue pressure changes recorded at a depth of 1 cm from the wall of a trochanteric bedsore cavity (with vertical wall) exposed to increasing levels of suction from a NPWT dressing.

Suction pressure (mmHg)	Tissue Pressure (mmHg)								
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Mean	SD	Min	Max
0	0	0	0	0	0	0	0.00	0	0
-75	1	2	1	1	1	1.2	0.45	1	2
-150	3	2	2	2	2	2.2	0.45	2	3
-225	4	3	3	3	3	3.2	0.45	3	4
-300	4	3	3	3	3	3.2	0.45	3	4
-375	4	4	3	3	4	3.6	0.55	3	4
-450	5	5	4	4	4	4.4	0.55	4	5

Table B.23: Tissue pressure changes recorded at a depth of 1 cm from the wall of a trochanteric bedsore cavity (with overhanging wall) exposed to increasing levels of suction from a NPWT dressing.

Suction pressure (mmHg)	Tissue Pressure (mmHg)								
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Mean	SD	Min	Max
0	0	0	0	0	0	0	0.00	0	0
-75	0	0	0	0	0	0	0.00	0	0
-150	0	1	1	1	1	0.8	0.45	0	1
-225	0	1	1	1	2	1	0.71	0	2
-300	0	1	1	1	2	1	0.71	0	2
-375	0	1	1	1	2	1	0.71	0	2
-450	0	1	1	1	2	1	0.71	0	2

Table B.24: Tissue pressure changes recorded at a depth of 1 cm from the wall of a trochanteric bedsore cavity (with bevelled wall) exposed to increasing levels of suction from a NPWT dressing.

Suction pressure (mmHg)	Tissue Pressure (mmHg)								
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Mean	SD	Min	Max
0	0	0	0	0	0	0	0.00	0	0
-75	10	4	1	5	6	5.2	3.27	1	10
-150	17	12	7	2	3	8.2	6.30	2	17
-225	25	11	8	5	4	10.6	8.50	4	25
-300	26	12	9	5	6	11.6	8.50	5	26
-375	26	12	9	6	7	12	8.15	6	26
-450	26	13	10	7	8	12.8	7.73	7	26

Table B.25: Tissue pressure changes recorded at a depth of 1 cm from the wall of a dehisced back wound cavity exposed to increasing levels of suction from a NPWT dressing.

Suction pressure (mmHg)	Tissue Pressure (mmHg)								
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Mean	SD	Min	Max
0	0	0	0	0	0	0	0.00	0	0
-75	-2	-5	-1	-1	1	-1.6	2.19	-5	1
-150	-1	2	-2	-2	-1	-0.8	1.64	-2	2
-225	-1	0	-2	0	0	-0.6	0.89	-2	0
-300	0	0	-1	0	2	0.2	1.10	-1	2
-375	1	-1	-1	1	3	0.6	1.67	-1	3
-450	1	0	0	2	4	1.4	1.67	0	4

Appendix C.
Experiment data: NPWT and perfusion - circumferential dressings

C1. Radioisotope studies

Table C.1: Perfusion in hands (as evidenced by radioactivity counts) and changes as a result of circumferential NPWT applied at two different suction pressures.

Volunteer	Hand	Suction Pressure (mmHg)	Radioactivity count (geometric mean of palmar and dorsal acquisitions)			
			No suction	Suction	Difference	Percentage reduction
1	Left	-400	13934	5674	8260	59.3
	Right	-400	14832	7713	7119	48.0
2	Left	-400	12706	6637	6069	47.8
	Right	-400	13605	8101	5504	40.5
3	Left	-400	12646	7580	5066	40.1
	Right	-400	17094	10938	6156	36.0
4	Left	-400	10817	5858	4959	45.8
	Right	-400	2226	1644	582	26.1
5	Left	-400	7908	4978	2930	37.1
	Right	-400	5334	4328	1006	18.9
6	Left	-125	14316	10163	4153	29.0
	Right	-125	15640	14223	1417	9.1
7	Left	-125	8524	7300	1224	14.4
	Right	-125	13786	12438	1348	9.8
8	Left	-125	8911	6214	2697	30.3
	Right	-125	10051	8141	1910	19
9	Left	-125	14089	19818	271	1.9
	Right	-125	12795	9980	2815	22
10	Left	-125	6339	5102	1237	19.5
	Right	-125	5515	4680	835	15.1

Appendix D.
Experiment data and demographics: NPWT and perfusion – non-circumferential dressings

D1. Transcutaneous partial pressure of oxygen studies

Table D.1: Volunteer demographics and mean reduction in perfusion (as evidenced by transcutaneous partial pressure of oxygen saturation ($tcpO_2$) changes) during periods of non-circumferential NPWT at two different suction pressures.

Volunteer	Sex	Age (yr)	Suction Pressure (mmHg)	Perfusion Reduction (%)	
				Left Leg	Right Leg
1	Female	35	-400	3.7	7.5
2	Female	32	-400	14.17	6.37
3	Male	35	-400	2.58	7.5
4	Female	35	-125	5.45	3.75
5	Female	29	-125	4	7.62
6	Male	35	-125	4.5	5.17

D1.1. Transcutaneous partial pressure of oxygen results

Table D.2: Transcutaneous partial pressure of oxygen saturation values (%) at specific time periods, during on-and off periods of suction at two different suction pressures (-400 mmHg and -125 mmHg) during non-circumferential NPWT.

Volunteer	Leg	Suction Pressure (mmHg	Time period (minutes)																			
			0	5	10	15	16	20	25	30	31	35	40	45	46	50	55	60	61	65	70	75
1	Left	0	66	65	66	67					69	66	67	69					70	69	67	68
		-400					67	61	63	63					67	62	63	64				
	Right	0	75	79	82	82					80	75	76	81					77	77	81	77
		-400					81	73	72	69					73	70	66	64				
2	Left	0	71	70	73	73					61	63	61	64					57	60	60	57
		-400					66	55	48	48					51	48	46	38				
	Right	0	58	58	58	59					60	61	59	58					57	60	63	60
		-400					60	57	51	48					53	54	48	52				
3	Left	0	48	49	57	53					56	46	56	57					50	49	55	58
		-400					51	51	50	50					52	53	47	48				
	Right	0	75	79	82	82					80	75	76	81					77	77	81	77
		-400					81	73	72	69					73	70	66	64				

Table D.2 continued: Transcutaneous partial pressure of oxygen saturation values (%) at specific time periods, during on-and off periods of suction at two different suction pressures (-400 mmHg and -125 mmHg) during non-circumferential NPWT.

Volunteer	Leg	Suction Pressure (mmHg	Time period (minutes)																			
			0	5	10	15	16	20	25	30	31	35	40	45	46	50	55	60	61	65	70	75
4	Left	0	70	74	67	72					64	64	63	65					63	64	63	67
		-125					64	61	60	58					63	61	60	60				
	Right	0	70	71	68	67					62	59	58	60					61	62	62	62
		-125					65	62	60	58					58	57	59	59				
5	Left	0	56	62	62	58					53	62	59	58					54	53	54	56
		-125					58	51	52	53					55	52	53	52				
	Right	0	53	57	52	54					52	49	49	54					51	50	58	51
		-125					49	47	47	45					31	46	45	49				
6	Left	0	65	67	65	67					64	64	66	65					73	69	72	70
		-125					63	61	60	62					57	64	67	68				
	Right	0	58	58	60	60					54	51	53	52					57	61	61	61
		-125					59	54	52	51					45	52	51	52				

Appendix E.
Experiment data and demographics: NPWT and perfusion – perfusion of surface area surrounding dressing

E1. Thermography studies

E1.1. Volunteer demographics

Table E.1: Volunteer demographics and suction pressure used on both sides of the lower back, in thermographic evaluation of perfusion changes around a NPWT dressing.

Volunteer	Sex	Age (yr)	Suction pressure (mmHg)
1	Male	22	Control
2	Female	23	Control
3	Male	22	-75
4	Female	24	-75
5	Female	23	-125
6	Male	29	-125
7	Male	29	-400
8	Male	38	-400

E1.2. Thermography results

Table E.2: Volunteer 1 Left side. Temperature values (°C) at different time periods of NPWT dressing application (with and without suction at -75 mmHg).

Distance from foam (mm)	Time Periods							
	Pre-NPWT	Dressing on					Dressing off	
		Suction off	Suction on		Suction off		5 min	5 min
	Baseline	Baseline	1 min	5 min	1 min	5 min		
1	32.76	32.52	32.77	32.42	31.37	31.10	33.82	33.68
2	32.82	32.63	32.72	32.86	32.47	32.47	33.69	33.64
3	32.74	32.75	32.75	32.86	32.65	32.90	33.61	33.64
4	32.72	32.76	32.78	32.88	32.94	33.02	33.52	33.62
5	32.70	32.76	32.78	32.85	33.01	33.10	33.41	33.56
6	32.74	32.79	32.78	32.87	33.05	33.15	33.43	33.56
7	32.76	32.82	32.81	32.92	33.05	33.17	33.52	33.57
8	32.82	32.90	32.84	32.92	33.01	33.12	33.50	33.56
9	32.88	32.99	32.88	32.94	33.03	33.14	33.44	33.56
10	32.90	33.01	32.90	32.98	33.09	33.18	33.41	33.57
11	32.91	33.01	32.91	33.01	33.14	33.23	33.36	33.54
12	32.94	33.03	32.93	33.03	33.14	33.27	33.34	33.52
13	32.98	33.00	32.97	33.05	33.07	33.29	33.35	33.50
14	32.99	32.97	32.95	33.01	33.05	33.22	33.33	33.47
15	32.98	32.99	32.93	32.99	33.05	33.20	33.33	33.45
16	32.96	32.97	32.94	33.02	33.02	33.17	33.33	33.42
17	32.94	32.94	32.94	33.05	33.02	33.15	33.30	33.35
18	32.98	32.93	32.95	33.05	33.05	33.14	33.28	33.27
19	33.03	32.92	32.99	33.05	33.06	33.13	33.25	33.26
20	33.01	32.91	32.98	33.04	33.03	33.10	33.22	33.28
21	32.96	32.94	32.95	32.99	33.01	33.05	33.21	33.25
22	32.92	33.02	33.01	32.97	33.03	33.01	33.22	33.26
23	32.97	33.03	33.09	33.00	33.06	33.01	33.21	33.29
24	32.97	33.01	33.13	33.07	33.14	33.10	33.20	33.32
25	32.93	32.99	33.13	33.14	33.23	33.15	33.19	33.31
26	32.92	32.99	33.11	33.15	33.24	33.14	33.15	33.25
27	32.92	33.01	33.11	33.21	33.19	33.16	33.16	33.26
28	32.90	33.04	33.09	33.26	33.21	33.19	33.23	33.33
29	32.89	32.76	33.13	33.28	33.23	33.24	33.28	33.36
30	32.87	33.02	33.14	33.26	33.24	33.29	33.26	33.34

Table E.2 continued: Volunteer 1 Left side. Temperature values (°C) at different time periods of NPWT dressing application (with and without suction at -75 mmHg).

Distance from foam (mm)	Time Periods							
	Pre-NPWT	Dressing on					Dressing off	
		Suction off	Suction on		Suction off		5 min	5 min
	Baseline	Baseline	1 min	5 min	1 min	5 min		
31	32.84	33.03	33.12	33.26	33.24	33.29	33.26	33.36
32	32.80	33.05	33.12	33.26	33.24	33.26	33.28	33.40
33	32.78	33.09	33.11	33.25	33.23	33.24	33.28	33.40
34	32.86	33.12	33.13	33.17	33.25	33.25	33.29	33.35
35	32.90	33.13	33.18	33.22	33.30	33.25	33.27	33.33
36	32.93	33.16	33.20	33.24	33.30	33.21	33.24	33.38
37	32.99	33.16	33.20	33.22	33.28	33.21	33.25	33.41
38	33.05	33.17	33.20	33.23	33.27	33.24	33.22	33.42
39	33.11	33.14	33.22	33.24	33.28	33.25	33.26	33.40
40	33.12	33.10	33.22	33.24	33.27	33.25	33.28	33.39
41	33.10	33.08	33.22	33.26	33.30	33.26	33.31	33.39
42	33.19	33.11	33.27	33.29	33.30	33.30	33.29	33.38
43	33.21	33.16	33.27	33.32	33.31	33.32	33.32	33.44
44	33.16	33.15	33.22	33.30	33.33	33.30	33.31	33.49
45	33.12	33.12	33.23	33.25	33.33	33.25	33.28	33.50
46	33.19	33.18	33.32	33.28	33.33	33.31	33.27	33.56
47	33.24	33.26	33.32	33.35	33.26	33.37	33.28	33.59
48	33.26	33.27	33.26	33.38	33.36	33.38	33.27	33.61
49	33.26	33.30	33.31	33.41	33.37	33.40	33.28	33.62
50	33.27	33.32	33.38	33.43	33.43	33.40	33.35	33.65
51	33.30	33.36	33.41	33.43	33.49	33.40	33.40	33.72
52	33.30	33.40	33.43	33.47	33.55	33.45	33.40	33.73
53	33.27	33.42	33.47	33.49	33.57	33.49	33.44	33.75
54	33.29	33.44	33.49	33.50	33.54	33.52	33.53	33.78
55	33.34	33.41	33.49	33.52	33.54	33.59	33.57	33.77
56	33.35	33.41	33.48	33.52	33.54	33.63	33.59	33.73
57	33.31	33.37	33.48	33.54	33.56	33.63	33.62	33.73
58	33.29	33.39	33.51	33.57	33.59	33.61	33.63	33.78
59	33.24	33.42	33.52	33.61	33.59	33.63	33.67	33.80
60	33.21	33.46	33.53	33.62	33.59	33.68	33.70	33.78

Table E.3: Volunteer 1 Right side. Temperature values (°C) at different time periods of NPWT dressing application (with and without suction at -75 mmHg).

Distance from foam (mm)	Time Periods							
	Pre-NPWT	Dressing on					Dressing off	
		Suction off	Suction on		Suction off		5 min	5 min
	Baseline	Baseline	1 min	5 min	1 min	5 min		
1	33.82	31.11	31.14	33.20	32.58	32.03	35.18	34.69
2	33.84	31.07	32.14	33.38	33.35	32.99	35.10	34.63
3	33.80	31.19	32.94	33.46	33.53	33.46	34.99	34.60
4	33.79	31.28	33.03	33.45	33.71	33.51	34.89	34.54
5	33.80	31.43	33.15	33.44	33.75	33.56	34.82	34.49
6	33.83	31.61	33.24	33.43	33.72	33.54	34.73	34.51
7	33.81	32.13	33.33	33.44	33.71	33.55	34.62	34.49
8	33.80	33.08	33.38	33.39	33.68	33.53	34.47	34.40
9	33.80	33.34	33.39	33.36	33.62	33.52	34.32	34.31
10	33.75	33.49	33.39	33.39	33.57	33.53	34.18	34.25
11	33.71	33.54	33.42	33.43	33.58	33.54	34.02	34.20
12	33.74	33.59	33.45	33.48	33.58	33.54	33.94	34.22
13	33.77	33.63	33.52	33.49	33.55	33.51	33.94	34.27
14	33.76	33.63	33.58	33.47	33.51	33.44	33.94	34.28
15	33.75	33.65	33.57	33.49	33.49	33.42	33.95	34.23
16	33.75	33.68	33.57	33.48	33.49	33.40	33.87	34.18
17	33.84	33.71	33.62	33.47	33.47	33.38	33.76	34.10
18	33.76	33.76	33.66	33.49	33.47	33.38	33.69	33.97
19	33.76	33.77	33.68	33.52	33.49	33.39	33.62	33.90
20	33.78	33.81	33.73	33.56	33.49	33.41	33.56	33.81
21	33.80	33.84	33.75	33.59	33.50	33.41	33.52	33.74
22	33.84	33.87	33.75	33.64	33.53	33.44	33.53	33.69
23	33.85	33.90	33.76	33.66	33.54	33.45	33.53	33.67
24	33.88	33.92	33.82	33.66	33.56	33.41	33.47	33.67
25	33.89	33.93	33.84	33.71	33.57	33.47	33.45	33.67
26	33.86	33.95	33.82	33.79	33.61	33.49	33.46	33.67
27	33.86	33.95	33.84	33.82	33.67	33.48	33.49	33.68
28	33.89	33.92	33.89	33.80	33.70	33.51	33.50	33.69
29	33.91	33.96	33.92	33.77	33.65	33.57	33.52	33.69
30	33.91	33.99	33.92	33.75	33.61	33.59	33.53	33.68
31	33.94	33.95	33.89	33.77	33.63	33.56	33.53	33.68
32	33.96	33.94	33.89	33.77	33.65	33.58	33.57	33.68
33	33.93	33.95	33.86	33.76	33.63	33.60	33.60	33.69

Table E.3 continued: Volunteer 1 Right side. Temperature values (°C) at different time periods of NPWT dressing application (with and without suction at -75 mmHg).

Distance from foam (mm)	Time Periods							
	Pre-NPWT	Dressing on					Dressing off	
		Suction off	Suction on		Suction off		5 min	5 min
	Baseline	Baseline	1 min	5 min	1 min	5 min		
34	33.91	33.96	33.80	33.75	33.61	33.62	33.64	33.71
35	33.91	33.96	33.77	33.78	33.60	33.63	33.68	33.73
36	33.91	33.92	33.77	33.80	33.61	33.65	33.70	33.71
37	33.90	33.91	33.78	33.80	33.64	33.66	33.68	33.67
38	33.88	33.93	33.79	33.78	33.67	33.67	33.64	33.64
39	33.90	33.98	33.81	33.79	33.67	33.67	33.63	33.64
40	33.94	33.99	33.83	33.80	33.67	33.65	33.64	33.67
41	33.93	33.98	33.83	33.83	33.70	33.63	33.64	33.69
42	33.89	33.99	33.84	33.85	33.73	33.67	33.63	33.69
43	33.89	34.01	33.84	33.84	33.72	33.69	33.61	33.72
44	33.89	34.02	33.85	33.84	33.75	33.67	33.64	33.71
45	33.92	33.96	33.88	33.89	33.78	33.67	33.66	33.69
46	33.97	34.00	33.92	33.92	33.75	33.70	33.69	33.71
47	34.00	34.05	33.96	33.89	33.74	33.68	33.71	33.72
48	34.03	34.03	33.96	33.85	33.76	33.67	33.73	33.73
49	34.04	34.03	33.95	33.85	33.76	33.70	33.73	33.74
50	34.05	34.05	33.94	33.85	33.75	33.68	33.70	33.73
51	34.04	34.06	33.92	33.84	33.75	33.63	33.69	33.73
52	34.06	34.05	33.93	33.83	33.75	33.62	33.70	33.72
53	34.04	34.03	33.96	33.84	33.72	33.63	33.74	33.73
54	33.99	34.01	33.95	33.83	33.69	33.66	33.76	33.75
55	33.94	33.99	33.92	33.79	33.71	33.65	33.74	33.75
56	33.96	33.99	33.91	33.80	33.71	33.65	33.74	33.76
57	33.96	34.00	33.90	33.84	33.68	33.65	33.73	33.73
58	33.94	33.98	33.89	33.85	33.68	33.65	33.67	33.69
59	33.94	34.02	33.89	33.84	33.68	33.64	33.67	33.71
60	33.92	34.04	33.86	33.80	33.67	33.63	33.69	33.71

Table E.4: Volunteer 2 Left side. Temperature values (°C) at different time periods of NPWT dressing application (with and without suction at -75 mmHg).

Distance from foam (mm)	Time Periods							
	Pre-NPWT	Dressing on					Dressing off	
		Suction off	Suction on		Suction off		5 min	5 min
	Baseline	Baseline	1 min	5 min	1 min	5 min		
1	34.28	28.90	31.86	32.67	32.28	31.20	35.38	34.90
2	34.21	29.25	32.02	32.96	32.74	32.01	35.26	34.81
3	34.11	30.13	32.50	33.39	33.24	32.87	35.09	34.73
4	34.04	31.03	32.89	33.34	33.37	33.50	34.92	34.64
5	33.99	32.14	33.11	33.47	33.67	34.00	34.76	34.57
6	33.92	33.02	33.28	33.50	33.82	34.00	34.62	34.49
7	33.85	33.06	33.41	33.54	33.84	34.10	34.54	34.39
8	33.78	33.26	33.50	33.60	33.85	34.19	34.45	34.29
9	33.73	33.37	33.59	33.59	33.92	34.28	34.33	34.21
10	33.71	33.47	33.66	33.52	33.95	34.24	34.30	34.14
11	33.78	33.53	33.68	33.55	33.89	34.14	34.30	34.09
12	33.85	33.55	33.67	33.60	33.83	34.10	34.23	34.02
13	33.90	33.55	33.69	33.62	33.75	34.02	34.15	33.98
14	33.97	33.56	33.73	33.65	33.67	33.93	34.12	33.98
15	34.00	33.60	33.73	33.65	33.62	33.89	34.10	33.98
16	34.02	33.66	33.74	33.64	33.57	33.83	34.07	33.97
17	34.07	33.72	33.74	33.62	33.56	33.80	34.00	33.93
18	34.07	33.73	33.73	33.63	33.55	33.75	33.97	33.88
19	34.06	33.72	33.76	33.66	33.49	33.72	33.98	33.86
20	34.13	33.73	33.75	33.66	33.46	33.69	33.89	33.86
21	34.11	33.70	33.74	33.62	33.43	33.63	33.86	33.82
22	34.09	33.67	33.71	33.58	33.42	33.54	33.85	33.76
23	34.09	33.67	33.64	33.55	33.40	33.49	33.82	33.76
24	34.09	33.65	33.61	33.51	33.35	33.46	33.75	33.75
25	34.00	33.60	33.58	33.50	33.33	33.45	33.70	33.70
26	33.94	33.56	33.53	33.54	33.37	33.49	33.72	33.67
27	33.97	33.56	33.59	33.59	33.44	33.49	33.74	33.65
28	33.94	33.60	33.62	33.62	33.49	33.49	33.76	33.63
29	33.88	33.63	33.62	33.64	33.52	33.49	33.77	33.63
30	33.86	33.68	33.64	33.65	33.52	33.51	33.77	33.61
31	33.87	33.72	33.70	33.67	33.52	33.51	33.76	33.57
32	33.91	33.74	33.74	33.71	33.52	33.51	33.75	33.58
33	33.89	33.75	33.74	33.72	33.55	33.54	33.74	33.60

Table E.4 continued: Volunteer 2 Left side. Temperature values (°C) at different time periods of NPWT dressing application (with and without suction at -75 mmHg).

Distance from foam (mm)	Time Periods							
	Pre-NPWT	Dressing on					Dressing off	
		Suction off	Suction on		Suction off		5 min	5 min
	Baseline	Baseline	1 min	5 min	1 min	5 min		
34	33.85	33.75	33.74	33.73	33.55	33.56	33.72	33.58
35	33.90	33.77	33.78	33.74	33.52	33.61	33.72	33.57
36	33.94	33.81	33.83	33.77	33.56	33.64	33.76	33.59
37	33.99	33.87	33.87	33.81	33.62	33.67	33.78	33.62
38	34.06	33.93	33.91	33.86	33.64	33.67	33.80	33.67
39	34.11	33.93	33.91	33.85	33.64	33.64	33.82	33.67
40	34.17	33.91	33.90	33.84	33.62	33.61	33.79	33.66
41	34.20	33.94	33.90	33.85	33.62	33.62	33.74	33.67
42	34.16	33.97	33.95	33.85	33.66	33.63	33.72	33.68
43	34.16	33.98	33.99	33.88	33.68	33.67	33.70	33.67
44	34.20	33.98	33.97	33.96	33.70	33.70	33.70	33.67
45	34.22	33.97	34.02	34.00	33.70	33.72	33.67	33.64
46	34.22	33.99	34.08	34.05	33.72	33.75	33.65	33.63
47	34.21	34.06	34.15	34.15	33.82	33.79	33.67	33.65
48	34.21	34.14	34.20	34.21	33.90	33.82	33.68	33.64
49	34.23	34.20	34.27	34.28	33.95	33.87	33.70	33.64
50	34.24	34.27	34.35	34.34	34.11	33.92	33.75	33.67
51	34.24	34.31	34.39	34.39	34.12	33.83	33.82	33.71
52	34.32	34.32	34.36	34.38	33.97	33.27	33.82	33.77
53	34.39	33.94	34.18	34.23	33.77	32.74	33.89	33.84
54	34.44	33.51	33.45	33.65	33.00	32.81	33.74	33.79
55	34.37	32.90	32.96	33.02	32.87	33.19	33.03	33.58
56	34.04	32.88	32.99	33.01	32.95	33.13	32.95	32.76
57	33.10	33.02	33.10	33.11	33.08	33.13	32.97	32.69
58	33.12	33.20	33.18	33.20	33.10	33.18	33.11	32.76
59	33.21	33.17	33.18	33.20	33.10	33.30	33.12	32.90
60	33.29	33.19	33.29	33.17	33.30	33.25	33.12	32.93

Table E.5: Volunteer 2 Right side. Temperature values (°C) at different time periods of NPWT dressing application (with and without suction at -75 mmHg).

Distance from foam (mm)	Time Periods							
	Pre-NPWT	Dressing on					Dressing off	
		Suction off	Suction on		Suction off		5 min	5 min
	Baseline	Baseline	1 min	5 min	1 min	5 min		
1	34.58	33.14	33.27	33.03	32.43	33.51	35.21	34.76
2	34.63	33.99	34.07	33.93	33.37	33.93	35.16	34.74
3	34.64	34.09	34.12	34.04	34.01	33.92	35.11	34.74
4	34.58	34.34	34.19	34.20	34.03	33.93	34.99	34.75
5	34.62	34.45	34.21	34.16	34.05	33.89	34.89	34.73
6	34.66	34.44	34.20	34.13	34.01	33.89	34.77	34.71
7	34.63	34.39	34.23	34.13	33.99	33.87	34.60	34.73
8	34.65	34.36	34.21	34.12	33.95	33.87	34.47	34.74
9	34.67	34.36	34.22	34.10	33.90	33.89	34.38	34.71
10	34.60	34.37	34.18	34.07	33.86	33.90	34.31	34.67
11	34.56	34.31	34.14	34.05	33.84	33.90	34.24	34.64
12	34.54	34.24	34.14	34.01	33.82	33.90	34.22	34.61
13	34.49	34.26	34.13	33.98	33.79	33.87	34.22	34.59
14	34.50	34.27	34.12	34.00	33.79	33.86	34.24	34.62
15	34.51	34.26	34.07	34.00	33.80	33.86	34.21	34.67
16	34.49	34.29	34.07	33.97	33.76	33.79	34.14	34.63
17	34.46	34.31	34.07	33.96	33.73	33.75	34.11	34.58
18	34.44	34.33	34.07	33.98	33.74	33.73	34.10	34.54
19	34.42	34.34	34.15	34.00	33.76	33.72	34.05	34.50
20	34.36	34.36	34.19	34.03	33.77	33.74	33.99	34.40
21	34.33	34.40	34.14	34.04	33.77	33.79	33.92	34.26
22	34.32	34.45	34.13	34.02	33.75	33.83	33.87	34.18
23	34.32	34.54	34.14	34.02	33.76	33.85	33.84	34.12
24	34.35	34.47	34.16	34.00	33.75	33.86	33.86	34.05
25	34.36	34.55	34.14	33.96	33.70	33.88	33.89	34.01
26	34.32	34.64	34.16	33.97	33.72	33.89	33.93	33.99
27	34.27	34.65	34.21	33.98	33.73	33.90	33.98	33.96
28	34.29	34.66	34.24	33.99	33.76	33.98	34.02	34.00
29	34.30	34.64	34.29	34.04	33.81	34.09	34.02	34.05
30	34.28	34.62	34.39	34.11	33.91	34.13	34.05	34.05
31	34.37	34.63	34.47	34.17	34.01	34.12	34.04	34.06
32	34.47	34.57	34.47	34.21	34.04	34.14	34.05	34.05
33	34.52	34.56	34.42	34.23	34.05	34.16	34.08	34.03

Table E.5 continued: Volunteer 2 Right side. Temperature values (°C) at different time periods of NPWT dressing application (with and without suction at -75 mmHg).

Distance from foam (mm)	Time Periods							
	Pre-NPWT	Dressing on					Dressing off	
		Suction off	Suction on		Suction off		5 min	5 min
	Baseline	Baseline	1 min	5 min	1 min	5 min		
34	34.57	34.58	34.39	34.23	34.07	34.22	34.08	34.02
35	34.54	34.59	34.41	34.21	34.01	34.55	34.05	34.01
36	34.51	34.60	34.41	34.21	33.93	34.36	34.08	34.03
37	34.51	34.63	34.40	34.20	33.94	34.40	34.12	34.07
38	34.51	34.67	34.42	34.19	34.01	34.73	34.17	34.09
39	34.52	34.69	34.45	34.20	34.07	34.63	34.23	34.11
40	34.54	34.69	34.47	34.23	34.10	34.71	34.30	34.15
41	34.60	34.71	34.48	34.23	34.12	34.79	34.33	34.25
42	34.64	34.68	34.46	34.26	34.13	34.83	34.38	34.36
43	34.65	34.73	34.46	34.21	34.12	34.68	34.50	34.49
44	34.65	34.68	34.50	34.21	34.13	33.73	34.62	34.63
45	34.70	34.50	34.51	34.25	34.18	33.73	34.69	34.73
46	34.74	33.73	34.42	34.30	34.19	33.67	34.79	34.81
47	34.77	33.61	33.84	34.35	33.98	33.82	34.77	34.88
48	34.76	33.60	33.15	34.37	33.02	33.80	34.22	34.38
49	34.34	33.85	32.58	33.75	33.02	33.32	33.61	33.61
50	33.83	33.79	32.71	33.00	33.19	33.37	33.63	33.61
51	33.51	33.29	33.04	33.00	33.26	33.41	33.72	33.53
52	33.60	33.76	33.20	32.93	33.32	33.08	33.84	33.65
53	33.79	33.82	32.41	33.19	32.57	32.45	33.79	33.63
54	33.94	33.31	32.51	33.25	32.72	32.01	33.51	33.08
55	33.45	32.51	33.39	32.38	33.33	31.96	33.41	33.08
56	33.81	32.00	33.25	32.96	32.93	31.94	33.51	33.11
57	33.89	32.07	31.97	33.47	32.03	32.11	33.04	32.63
58	33.90	32.55	31.03	33.33	31.30	32.90	32.30	31.80
59	33.02	33.36	30.99	32.38	31.33	33.33	31.97	31.42
60	32.42	33.43	31.01	31.77	31.36	33.42	32.05	31.42

Table E.6: Volunteer 3 Left side. Temperature values (°C) at different time periods of NPWT dressing application (with and without suction at -125 mmHg).

Distance from foam (mm)	Time Periods							
	Pre-NPWT	Dressing on					Dressing off	
		Suction off	Suction on		Suction off		5 min	5 min
	Baseline	Baseline	1 min	5 min	1 min	5 min		
1	34.61	30.41	33.35	33.11	30.28	31.07	35.45	35.45
2	34.60	30.55	34.11	34.15	31.33	31.91	35.35	35.39
3	34.59	30.69	33.95	34.58	32.35	33.09	35.28	35.37
4	34.63	30.83	33.98	34.56	33.54	34.01	35.19	35.37
5	34.62	31.26	34.14	34.57	34.41	34.62	35.08	35.36
6	34.61	31.46	34.31	34.59	34.42	34.66	34.98	35.31
7	34.61	31.43	34.36	34.58	34.57	34.76	34.91	35.22
8	34.57	31.60	34.34	34.54	34.63	34.76	34.86	35.20
9	34.58	32.16	34.35	34.55	34.64	34.73	34.82	35.13
10	34.58	32.46	34.38	34.60	34.62	34.73	34.80	35.09
11	34.55	33.07	34.43	34.64	34.61	34.75	34.77	35.08
12	34.56	33.78	34.50	34.69	34.59	34.76	34.77	35.07
13	34.61	34.21	34.51	34.73	34.59	34.75	34.77	35.05
14	34.67	34.31	34.51	34.74	34.62	34.78	34.75	35.04
15	34.67	34.39	34.52	34.72	34.64	34.78	34.74	35.06
16	34.65	34.35	34.48	34.70	34.63	34.77	34.72	35.03
17	34.64	34.35	34.47	34.69	34.60	34.74	34.71	35.00
18	34.63	34.42	34.49	34.69	34.59	34.73	34.69	34.98
19	34.71	34.48	34.51	34.67	34.57	34.73	34.70	34.94
20	34.79	34.48	34.54	34.68	34.54	34.74	34.73	34.94
21	34.83	34.49	34.59	34.70	34.57	34.75	34.74	34.96
22	34.86	34.51	34.60	34.68	34.60	34.73	34.72	34.93
23	34.85	34.51	34.61	34.66	34.59	34.67	34.67	34.91
24	34.86	34.56	34.64	34.67	34.55	34.62	34.67	34.94
25	34.87	34.63	34.69	34.70	34.52	34.60	34.70	34.96
26	34.88	34.70	34.76	34.74	34.49	34.59	34.68	34.95
27	34.90	34.78	34.81	34.80	34.51	34.60	34.67	34.93
28	34.90	34.83	34.86	34.86	34.57	34.64	34.67	34.93
29	34.88	34.91	34.95	34.92	34.65	34.67	34.69	34.95
30	34.92	34.96	34.99	34.96	34.68	34.71	34.68	34.96
31	34.95	34.98	35.02	34.97	34.73	34.72	34.67	34.95
32	34.96	35.03	35.03	34.94	34.79	34.72	34.67	34.94
33	34.98	35.06	34.99	34.94	34.79	34.74	34.67	34.93

Table E.6 continued: Volunteer 3 Left side. Temperature values (°C) at different time periods of NPWT dressing application (with and without suction at -125 mmHg).

Distance from foam (mm)	Time Periods							
	Pre-NPWT	Dressing on					Dressing off	
		Suction off	Suction on		Suction off		5 min	5 min
	Baseline	Baseline	1 min	5 min	1 min	5 min		
34	35.00	35.04	34.95	34.95	34.75	34.73	34.68	34.96
35	34.98	35.01	34.92	34.94	34.75	34.69	34.68	34.98
36	34.95	35.05	34.92	34.92	34.72	34.69	34.68	34.96
37	34.97	35.10	34.97	34.91	34.68	34.69	34.74	34.93
38	35.02	35.15	34.99	34.92	34.68	34.66	34.78	34.86
39	35.07	35.17	35.02	34.92	34.69	34.65	34.79	34.84
40	35.10	35.18	35.05	34.97	34.72	34.70	34.82	34.80
41	35.13	35.22	35.13	35.02	34.74	34.76	34.84	34.78
42	35.15	35.23	35.17	35.06	34.76	34.79	34.82	34.79
43	35.14	35.22	35.13	35.07	34.80	34.81	34.79	34.80
44	35.16	35.23	35.13	35.06	34.85	34.82	34.81	34.83
45	35.15	35.24	35.13	35.05	34.84	34.83	34.79	34.84
46	35.11	35.23	35.13	35.02	34.81	34.82	34.73	34.84
47	35.06	35.25	35.17	35.02	34.82	34.81	34.75	34.84
48	35.06	35.27	35.21	35.03	34.80	34.83	34.77	34.83
49	35.11	35.24	35.21	35.04	34.80	34.82	34.77	34.85
50	35.11	35.23	35.21	35.08	34.85	34.87	34.78	34.94
51	35.10	35.24	35.24	35.10	34.88	34.89	34.79	35.02
52	35.10	35.26	35.23	35.11	34.88	34.87	34.80	35.06
53	35.11	35.25	35.23	35.11	34.87	34.86	34.80	35.12
54	35.13	35.25	35.24	35.10	34.85	34.87	34.81	35.15
55	35.11	35.27	35.21	35.13	34.85	34.90	34.83	35.14
56	35.11	35.27	35.23	35.16	34.89	34.92	34.84	35.12
57	35.11	35.23	35.26	35.13	34.91	34.94	34.84	35.10
58	35.07	35.20	35.31	35.12	34.92	34.95	34.87	35.07
59	35.07	35.20	35.30	35.12	34.93	34.95	34.86	35.08
60	35.11	35.19	35.26	35.12	34.93	34.96	34.82	35.12

Table E.7: Volunteer 3 Right side. Temperature values (°C) at different time periods of NPWT dressing application (with and without suction at -125 mmHg).

Distance from foam (mm)	Time Periods							
	Pre-NPWT	Dressing on					Dressing off	
		Suction off	Suction on		Suction off		5 min	5 min
	Baseline	Baseline	1 min	5 min	1 min	5 min		
1	35.36	33.75	34.52	34.54	31.59	32.07	35.86	35.69
2	35.34	34.43	34.64	34.94	32.78	33.46	35.82	35.70
3	35.28	34.60	34.75	34.92	34.15	34.55	35.71	35.66
4	35.19	34.72	34.73	34.88	34.74	34.73	35.55	35.64
5	35.13	34.77	34.71	34.86	34.80	34.88	35.43	35.62
6	35.10	34.77	34.71	34.85	34.82	34.90	35.33	35.54
7	35.06	34.75	34.67	34.73	34.83	34.92	35.21	35.48
8	35.02	34.72	34.65	34.69	34.83	34.92	35.11	35.47
9	34.95	34.71	34.67	34.67	34.80	34.90	35.01	35.47
10	34.91	34.73	34.66	34.66	34.75	34.88	34.87	35.45
11	34.90	34.72	34.64	34.63	34.70	34.83	34.79	35.39
12	34.92	34.69	34.68	34.64	34.66	34.78	34.79	35.35
13	34.90	34.74	34.75	34.72	34.64	34.78	34.81	35.31
14	34.88	34.75	34.80	34.75	34.64	34.78	34.82	35.23
15	34.86	34.73	34.80	34.73	34.62	34.72	34.84	35.20
16	34.90	34.72	34.80	34.73	34.58	34.65	34.83	35.22
17	34.92	34.71	34.79	34.72	34.55	34.61	34.82	35.22
18	34.90	34.73	34.78	34.66	34.54	34.61	34.79	35.15
19	34.90	34.75	34.73	34.61	34.52	34.59	34.72	35.11
20	34.89	34.74	34.71	34.59	34.50	34.56	34.67	35.12
21	34.85	34.77	34.70	34.58	34.47	34.54	34.66	35.08
22	34.81	34.82	34.71	34.59	34.43	34.52	34.63	35.00
23	34.78	34.79	34.70	34.57	34.41	34.48	34.58	34.92
24	34.74	34.77	34.69	34.57	34.40	34.50	34.56	34.81
25	34.74	34.81	34.68	34.55	34.40	34.51	34.52	34.72
26	34.73	34.84	34.65	34.50	34.40	34.45	34.49	34.65
27	34.75	34.87	34.69	34.54	34.40	34.46	34.49	34.63
28	34.81	34.97	34.79	34.65	34.38	34.48	34.53	34.55
29	34.85	35.10	34.90	34.78	34.39	34.51	34.61	34.52
30	34.89	35.22	34.99	34.91	34.48	34.58	34.70	34.51
31	34.97	35.29	35.09	35.00	34.58	34.66	34.79	34.52
32	35.07	35.35	35.19	35.11	34.65	34.74	34.87	34.54
33	35.19	35.42	35.33	35.22	34.77	34.87	34.95	34.58

Table E.7 continued: Volunteer 3 Right side. Temperature values (°C) at different time periods of NPWT dressing application (with and without suction at -125 mmHg).

Distance from foam (mm)	Time Periods							
	Pre-NPWT	Dressing on					Dressing off	
		Suction off	Suction on		Suction off		5 min	5 min
	Baseline	Baseline	1 min	5 min	1 min	5 min		
34	35.31	35.40	35.41	35.26	34.87	34.94	34.98	34.63
35	35.42	35.43	35.39	35.26	34.95	34.98	34.98	34.70
36	35.50	35.47	35.36	35.25	35.00	34.97	34.98	34.71
37	35.47	35.42	35.33	35.23	34.97	34.94	34.97	34.69
38	35.40	35.39	35.30	35.17	34.94	34.90	34.94	34.68
39	35.35	35.38	35.28	35.11	34.92	34.88	34.94	34.67
40	35.34	35.36	35.27	35.09	34.90	34.87	34.92	34.68
41	35.34	35.33	35.24	35.10	34.87	34.81	34.85	34.65
42	35.31	35.29	35.19	35.12	34.83	34.76	34.79	34.63
43	35.26	35.24	35.15	35.04	34.81	34.76	34.75	34.61
44	35.24	35.24	35.11	34.99	34.81	34.71	34.72	34.58
45	35.22	35.20	35.07	34.90	34.76	34.66	34.68	34.56
46	35.12	35.19	35.06	34.85	34.71	34.65	34.68	34.56
47	35.06	35.19	35.03	34.90	34.69	34.64	34.70	34.54
48	35.10	35.18	34.99	34.94	34.69	34.66	34.73	34.56
49	35.10	35.19	35.02	34.97	34.72	34.68	34.78	34.59
50	35.11	35.24	35.10	34.97	34.74	34.69	34.84	34.58
51	35.15	35.28	35.13	34.97	34.72	34.69	34.88	34.59
52	35.16	35.32	35.17	34.99	34.73	34.71	34.91	34.60
53	35.22	35.35	35.20	35.02	34.74	34.74	34.94	34.64
54	35.26	35.35	35.20	35.03	34.77	34.72	34.98	34.72
55	35.25	35.35	35.20	35.00	34.78	34.71	34.99	34.74
56	35.25	35.31	35.21	34.96	34.76	34.73	34.98	34.74
57	35.24	35.32	35.17	34.98	34.77	34.71	35.00	34.75
58	35.22	35.36	35.15	34.95	34.76	34.71	35.01	34.72
59	35.26	35.41	35.16	34.94	34.78	34.75	34.99	34.70
60	35.28	35.40	35.18	34.96	34.78	34.74	34.93	34.70

Table E.8: Volunteer 4 Left side. Temperature values (°C) at different time periods of NPWT dressing application (with and without suction at -125 mmHg).

Distance from foam (mm)	Time Periods							
	Pre-NPWT	Dressing on					Dressing off	
		Suction off	Suction on		Suction off		5 min	5 min
	Baseline	Baseline	1 min	5 min	1 min	5 min		
1	33.87	31.65	32.63	33.61	32.95	33.36	34.95	34.31
2	33.86	32.48	33.21	33.58	33.66	33.50	34.85	34.24
3	33.88	33.15	33.27	33.54	33.73	33.65	34.73	34.17
4	33.91	33.33	33.37	33.50	33.81	33.70	34.65	34.14
5	33.90	33.45	33.40	33.48	33.82	33.70	34.54	34.13
6	33.89	33.48	33.40	33.50	33.79	33.70	34.42	34.14
7	33.93	33.54	33.42	33.54	33.75	33.69	34.30	34.14
8	33.95	33.58	33.46	33.58	33.75	33.74	34.21	34.13
9	33.97	33.64	33.52	33.66	33.76	33.73	34.15	34.14
10	34.03	33.72	33.61	33.73	33.78	33.74	34.05	34.14
11	34.10	33.79	33.65	33.77	33.80	33.74	33.99	34.10
12	34.13	33.85	33.73	33.81	33.86	33.84	33.98	34.11
13	34.09	33.90	33.82	33.84	33.91	33.84	33.99	34.19
14	34.10	33.93	33.85	33.88	33.99	33.89	34.01	34.22
15	34.15	33.94	33.86	33.92	34.02	33.99	34.04	34.20
16	34.13	33.96	33.87	34.03	34.00	34.00	34.06	34.19
17	34.10	34.02	33.92	34.13	34.01	34.03	34.04	34.22
18	34.07	34.05	34.05	34.14	34.14	34.08	34.02	34.22
19	34.05	34.14	34.13	34.11	34.26	34.10	34.01	34.23
20	34.03	34.22	34.15	34.05	34.26	34.10	34.00	34.24
21	33.98	34.25	34.14	34.00	34.18	34.12	34.01	34.22
22	33.92	34.25	34.14	34.00	34.13	34.11	34.06	34.21
23	33.89	34.20	34.10	33.97	34.09	34.09	34.08	34.20
24	33.83	34.13	34.05	33.93	34.05	34.07	34.07	34.19
25	33.79	34.08	34.00	33.93	34.02	34.03	34.08	34.19
26	33.85	34.02	33.94	33.92	33.99	34.00	34.10	34.17
27	33.74	34.02	33.91	33.90	33.95	34.00	34.10	34.16
28	33.74	34.00	33.91	33.88	33.92	34.01	34.10	34.17
29	33.76	33.94	33.88	33.88	33.92	34.01	34.09	34.21
30	33.77	33.91	33.84	33.88	33.90	34.01	34.11	34.26
31	33.79	33.91	33.82	33.88	33.87	33.99	34.11	34.29
32	33.81	33.88	33.84	33.88	33.90	33.98	34.14	34.30
33	33.84	33.86	33.84	33.86	33.94	34.04	34.17	34.28

Table E.8 continued: Volunteer 4 Left side. Temperature values (°C) at different time periods of NPWT dressing application (with and without suction at -125 mmHg).

Distance from foam (mm)	Time Periods							
	Pre-NPWT	Dressing on					Dressing off	
		Suction off	Suction on		Suction off		5 min	5 min
	Baseline	Baseline	1 min	5 min	1 min	5 min		
34	33.86	33.87	33.80	33.86	33.96	34.06	34.17	34.23
35	33.89	33.88	33.78	33.90	33.95	34.02	34.15	34.16
36	33.89	33.89	33.80	33.93	33.95	33.96	34.15	34.14
37	33.87	33.89	33.82	33.94	33.95	33.94	34.16	34.14
38	33.91	33.94	33.83	33.95	33.95	33.88	34.18	34.12
39	33.96	33.95	33.84	33.99	33.94	33.81	34.19	34.09
40	33.98	33.96	33.84	33.99	33.94	33.78	34.24	34.10
41	33.98	33.98	33.83	33.99	33.94	33.75	34.34	34.14
42	33.96	33.98	33.81	34.03	33.86	33.71	34.40	34.16
43	33.97	34.01	33.83	34.03	33.97	33.76	34.41	34.16
44	33.98	34.02	33.86	34.04	34.02	33.82	34.44	34.15
45	33.96	34.05	33.87	34.01	34.06	33.84	34.46	34.14
46	33.95	34.06	33.88	33.99	34.04	33.82	34.43	34.12
47	33.94	34.06	33.92	34.02	34.01	33.81	34.40	34.12
48	33.91	34.05	33.92	34.03	33.99	33.77	34.36	34.16
49	33.90	34.01	33.92	34.00	34.01	33.76	34.33	34.14
50	33.89	34.02	33.90	33.96	34.01	33.81	34.36	34.15
51	33.88	34.02	33.86	34.00	34.02	33.86	34.40	34.21
52	33.89	34.00	33.86	34.00	34.03	33.88	34.38	34.25
53	33.89	34.00	33.86	33.96	33.99	33.89	34.38	34.23
54	33.94	34.01	33.86	33.94	33.96	33.89	34.42	34.23
55	33.96	34.01	33.86	33.96	33.95	33.84	34.44	34.24
56	33.94	34.01	33.82	33.95	33.90	33.82	34.46	34.26
57	33.93	33.98	33.82	33.97	33.89	33.81	34.48	34.26
58	33.93	33.98	33.81	34.05	33.92	33.79	34.45	34.28
59	33.93	33.99	33.84	34.09	33.97	33.74	34.40	34.30
60	33.88	34.01	33.89	34.04	33.98	33.70	34.35	34.28

Table E.9: Volunteer 4 Right side. Temperature values (°C) at different time periods of NPWT dressing application (with and without suction at -125 mmHg).

Distance from foam (mm)	Time Periods							
	Pre-NPWT	Dressing on					Dressing off	
		Suction off	Suction on		Suction off		5 min	5 min
	Baseline	Baseline	1 min	5 min	1 min	5 min		
1	33.99	32.37	32.10	33.52	34.20	33.82	34.86	34.26
2	33.90	32.95	32.57	33.66	34.32	34.36	34.75	34.23
3	33.81	32.97	32.91	33.70	34.43	34.33	34.56	34.23
4	33.77	33.04	33.10	33.64	34.37	34.31	34.38	34.26
5	33.72	33.08	33.10	33.52	34.25	34.26	34.26	34.30
6	33.67	33.11	33.05	33.45	34.09	34.19	34.19	34.37
7	33.61	33.17	33.00	33.38	34.01	34.10	34.11	34.42
8	33.58	33.22	32.99	33.39	33.97	34.03	34.07	34.34
9	33.56	33.27	33.04	33.39	33.91	33.96	34.04	34.26
10	33.55	33.30	33.08	33.39	33.85	33.91	34.00	34.20
11	33.60	33.29	33.14	33.39	33.83	33.89	33.95	34.15
12	33.65	33.32	33.19	33.37	33.82	33.87	33.96	34.07
13	33.63	33.37	33.22	33.40	33.75	33.86	34.01	34.01
14	33.62	33.37	33.28	33.42	33.69	33.91	34.07	34.03
15	33.63	33.40	33.31	33.40	33.69	33.90	34.13	34.07
16	33.62	33.46	33.31	33.41	33.71	33.91	34.19	34.09
17	33.63	33.49	33.31	33.51	33.76	33.92	34.23	34.12
18	33.57	33.52	33.40	33.60	33.76	33.88	34.22	34.14
19	33.60	33.57	33.49	33.56	33.71	33.86	34.21	34.18
20	33.63	33.63	33.53	33.57	33.69	33.86	34.19	34.26
21	33.62	33.65	33.53	33.58	33.73	33.85	34.15	34.33
22	33.63	33.68	33.49	33.59	33.79	33.85	34.12	34.37
23	33.67	33.76	33.49	33.65	33.85	33.93	34.07	34.38
24	33.72	33.84	33.55	33.71	33.88	34.03	34.05	34.36
25	33.79	33.92	33.62	33.76	33.95	34.06	34.04	34.34
26	33.90	33.98	33.67	33.84	34.06	34.08	34.01	34.31
27	34.01	34.07	33.72	33.97	34.16	34.08	33.98	34.28
28	34.12	34.14	33.83	34.08	34.24	34.02	33.98	34.28
29	34.21	34.17	33.97	34.16	34.28	33.94	34.04	34.26
30	34.27	34.15	34.12	34.23	34.31	33.94	34.08	34.22
31	34.27	34.13	34.18	34.22	34.29	33.94	34.12	34.22
32	34.22	34.10	34.17	34.16	34.22	33.90	34.10	34.22
33	34.11	34.07	34.17	34.12	34.16	33.85	34.17	34.20

Table E.9 continued: Volunteer 4 Right side. Temperature values (°C) at different time periods of NPWT dressing application (with and without suction at -125 mmHg).

Distance from foam (mm)	Time Periods							
	Pre-NPWT	Dressing on					Dressing off	
		Suction off	Suction on		Suction off		5 min	5 min
	Baseline	Baseline	1 min	5 min	1 min	5 min		
34	34.01	34.06	34.13	34.06	34.11	33.86	34.17	34.17
35	33.96	34.02	34.06	34.02	34.04	33.88	34.16	34.14
36	33.95	33.96	34.01	34.00	34.00	33.91	34.19	34.18
37	33.91	33.94	33.98	33.97	33.98	33.94	34.22	34.23
38	33.86	33.94	33.94	33.93	33.95	33.94	34.27	34.28
39	33.86	33.96	33.89	33.90	33.94	33.96	34.28	34.30
40	33.89	33.97	33.87	33.88	33.92	33.96	34.28	34.30
41	33.92	33.99	33.87	33.85	33.91	33.98	34.33	34.31
42	33.93	34.01	33.87	33.86	33.94	34.03	34.40	34.31
43	33.97	34.04	33.91	33.88	33.98	34.01	34.42	34.30
44	33.98	34.02	33.91	33.92	34.03	34.02	34.43	34.28
45	33.98	34.03	33.90	33.93	34.04	34.09	34.40	34.31
46	34.01	34.06	33.94	34.00	34.06	34.11	34.36	34.34
47	34.03	34.07	33.98	34.07	34.13	34.07	34.35	34.35
48	34.06	34.10	33.94	34.07	34.17	34.00	34.34	34.37
49	34.09	34.15	33.97	34.07	34.17	33.96	34.34	34.44
50	34.10	34.16	34.04	34.09	34.20	33.94	34.36	34.49
51	34.11	34.13	34.08	34.11	34.23	33.96	34.42	34.54
52	34.08	34.19	34.09	34.14	34.27	34.01	34.47	34.62
53	34.08	34.20	34.12	34.19	34.34	34.03	34.53	34.70
54	34.11	34.27	34.15	34.23	34.35	34.02	34.58	34.73
55	34.10	34.28	34.13	34.24	34.37	34.00	34.63	34.72
56	34.09	34.22	34.10	34.25	34.38	34.00	34.66	34.71
57	34.08	34.22	34.12	34.23	34.40	33.97	34.68	34.66
58	34.06	34.23	34.13	34.19	34.38	33.97	34.68	34.58
59	33.99	34.21	34.15	34.19	34.35	34.00	34.62	34.53
60	33.93	34.19	34.17	34.19	34.30	34.01	34.58	34.47

Table E.10: Volunteer 5 Left side. Temperature values (°C) at different time periods of NPWT dressing application (with and without suction at -400 mmHg).

Distance from foam (mm)	Time Periods							
	Pre-NPWT	Dressing on					Dressing off	
		Suction off	Suction on		Suction off		5 min	5 min
	Baseline	Baseline	1 min	5 min	1 min	5 min		
1	34.19	31.37	32.78	33.29	32.46	33.76	34.60	34.91
2	34.21	31.56	32.66	33.55	33.41	33.76	34.59	34.87
3	34.21	31.90	32.76	33.69	33.79	33.90	34.45	34.86
4	34.23	32.30	32.93	33.83	33.76	33.92	34.40	34.83
5	34.26	32.74	33.09	33.80	33.85	33.91	34.39	34.86
6	34.26	32.99	33.34	33.74	33.87	33.86	34.37	34.91
7	34.23	33.49	33.41	33.47	33.86	33.81	34.36	34.89
8	34.22	33.61	33.37	33.45	33.80	33.86	34.36	34.84
9	34.25	33.80	33.37	33.47	33.72	33.97	34.37	34.80
10	34.23	33.79	33.67	33.57	33.72	33.97	34.39	34.72
11	34.18	33.80	33.81	33.72	33.86	33.96	34.38	34.59
12	34.19	33.82	33.82	33.81	33.86	33.97	34.37	34.44
13	34.22	33.84	33.87	33.85	33.83	34.00	34.35	34.37
14	34.24	33.89	33.93	33.89	33.84	34.02	34.33	34.30
15	34.24	33.92	34.00	33.93	33.88	34.02	34.33	34.25
16	34.21	33.95	34.00	33.96	33.92	34.03	34.32	34.21
17	34.22	33.99	33.96	33.96	33.91	34.02	34.30	34.18
18	34.24	34.07	33.96	33.99	33.89	33.97	34.35	34.19
19	34.25	34.11	33.99	34.01	33.89	33.97	34.39	34.17
20	34.23	34.08	34.02	34.02	33.91	34.00	34.40	34.16
21	34.20	34.07	34.02	34.04	33.91	34.02	34.42	34.16
22	34.19	34.08	34.04	34.03	33.90	34.04	34.48	34.18
23	34.19	34.05	34.09	34.03	33.90	34.05	34.53	34.23
24	34.19	34.03	34.09	34.03	33.92	34.02	34.55	34.25
25	34.20	34.09	34.08	34.06	33.91	33.97	34.56	34.24
26	34.19	34.16	34.07	34.06	33.93	33.95	34.54	34.21
27	34.16	34.21	34.02	34.03	33.90	33.98	34.55	34.20
28	34.13	34.26	34.05	34.04	33.88	34.02	34.58	34.22
29	34.12	34.26	34.13	34.04	33.90	34.07	34.60	34.25
30	34.09	34.26	34.16	34.11	33.92	34.11	34.62	34.23
31	34.10	34.26	34.14	34.20	33.98	34.12	34.65	34.23
32	34.09	34.24	34.14	34.20	34.00	34.08	34.67	34.27
33	34.08	34.21	34.10	34.15	34.02	34.06	34.68	34.27

Table E.10 continued: Volunteer 5 Left side. Temperature values (°C) at different time periods of NPWT dressing application (with and without suction at -400 mmHg).

Distance from foam (mm)	Time Periods							
	Pre-NPWT	Dressing on					Dressing off	
		Suction off	Suction on		Suction off		5 min	5 min
	Baseline	Baseline	1 min	5 min	1 min	5 min		
34	34.06	34.18	34.07	34.15	34.02	34.06	34.70	34.30
35	34.02	34.21	34.10	34.13	34.00	34.01	34.71	34.31
36	33.98	34.19	34.09	34.08	33.98	33.97	34.67	34.30
37	33.97	34.15	34.07	34.08	33.97	33.98	34.61	34.30
38	34.01	34.16	34.08	34.08	33.94	34.05	34.57	34.27
39	34.01	34.19	34.09	34.08	33.91	34.07	34.56	34.25
40	33.98	34.23	34.14	34.10	33.92	34.04	34.58	34.24
41	33.99	34.24	34.16	34.13	33.94	34.01	34.53	34.21
42	33.96	34.28	34.15	34.13	33.93	34.02	34.46	34.16
43	33.95	34.33	34.18	34.13	33.91	34.08	34.40	34.17
44	34.00	34.37	34.22	34.13	33.90	34.14	34.38	34.17
45	34.04	34.42	34.25	34.18	33.90	34.19	34.33	34.13
46	34.07	34.47	34.19	34.23	33.95	34.24	34.26	34.13
47	34.10	34.51	34.23	34.24	34.01	34.28	34.26	34.15
48	34.12	34.51	34.33	34.25	34.05	34.30	34.22	34.09
49	34.15	34.55	34.39	34.27	34.12	34.31	34.15	34.04
50	34.19	34.56	34.42	34.28	34.15	34.31	34.13	34.01
51	34.23	34.56	34.45	34.29	34.13	34.33	34.12	34.04
52	34.28	34.54	34.48	34.34	34.19	34.31	34.09	34.11
53	34.28	34.48	34.48	34.38	34.23	34.33	34.10	34.11
54	34.27	34.44	34.42	34.38	34.23	34.30	34.12	34.07
55	34.24	34.45	34.34	34.35	34.19	34.21	34.11	34.09
56	34.23	34.47	34.32	34.32	34.11	34.18	34.11	34.13
57	34.17	34.48	34.33	34.30	34.07	34.21	34.11	34.21
58	34.09	34.48	34.30	34.30	34.04	34.22	34.09	34.25
59	34.09	34.49	34.33	34.31	34.04	34.19	34.06	34.26
60	34.16	34.49	34.38	34.31	34.05	34.21	34.07	34.28

Table E.11: Volunteer 5 Right side. Temperature values (°C) at different time periods of NPWT dressing application (with and without suction at -400 mmHg).

Distance from foam (mm)	Time Periods							
	Pre-NPWT	Dressing on					Dressing off	
		Suction off	Suction on		Suction off		5 min	5 min
	Baseline	Baseline	1 min	5 min	1 min	5 min		
1	33.67	31.14	32.05	33.33	32.59	31.50	34.77	34.53
2	33.69	32.22	33.19	33.41	33.23	32.54	34.77	34.60
3	33.69	32.92	33.17	33.50	33.31	33.30	34.75	34.63
4	33.77	33.33	33.25	33.47	33.41	33.32	34.64	34.63
5	33.69	33.37	33.26	33.45	33.42	33.36	34.51	34.62
6	33.63	33.46	33.31	33.41	33.42	33.39	34.38	34.56
7	33.64	33.48	33.35	33.38	33.35	33.44	34.23	34.46
8	33.67	33.46	33.34	33.34	33.28	33.48	34.09	34.32
9	33.63	33.47	33.27	33.32	33.27	33.48	33.98	34.15
10	33.56	33.48	33.25	33.38	33.34	33.46	33.85	33.99
11	33.50	33.50	33.35	33.43	33.42	33.47	33.72	33.85
12	33.51	33.55	33.42	33.43	33.46	33.46	33.65	33.74
13	33.50	33.56	33.42	33.45	33.45	33.44	33.60	33.68
14	33.49	33.54	33.44	33.47	33.42	33.41	33.54	33.61
15	33.49	33.55	33.48	33.47	33.44	33.34	33.55	33.55
16	33.51	33.55	33.48	33.47	33.43	33.38	33.60	33.46
17	33.51	33.54	33.48	33.47	33.39	33.41	33.58	33.46
18	33.49	33.54	33.49	33.47	33.37	33.37	33.53	33.46
19	33.51	33.54	33.51	33.51	33.35	33.37	33.50	33.43
20	33.49	33.57	33.52	33.55	33.39	33.37	33.47	33.46
21	33.47	33.60	33.53	33.54	33.42	33.34	33.46	33.50
22	33.48	33.60	33.56	33.54	33.42	33.37	33.48	33.49
23	33.46	33.58	33.60	33.56	33.41	33.37	33.47	33.50
24	33.50	33.54	33.60	33.55	33.42	33.33	33.40	33.51
25	33.52	33.55	33.56	33.53	33.41	33.27	33.35	33.50
26	33.47	33.54	33.53	33.51	33.35	33.25	33.39	33.49
27	33.49	33.53	33.53	33.52	33.39	33.31	33.39	33.51
28	33.56	33.56	33.58	33.56	33.45	33.39	33.36	33.51
29	33.57	33.59	33.60	33.57	33.48	33.46	33.30	33.50
30	33.56	33.60	33.62	33.58	33.48	33.49	33.31	33.45
31	33.56	33.62	33.58	33.59	33.49	33.52	33.32	33.45
32	33.53	33.65	33.58	33.58	33.49	33.50	33.28	33.50
33	33.47	33.60	33.63	33.59	33.49	33.49	33.27	33.53

Table E.11 continued: Volunteer 5 Right side. Temperature values (°C) at different time periods of NPWT dressing application (with and without suction at -400 mmHg).

Distance from foam (mm)	Time Periods							
	Pre-NPWT	Dressing on					Dressing off	
		Suction off	Suction on		Suction off		5 min	5 min
	Baseline	Baseline	1 min	5 min	1 min	5 min		
34	33.48	33.60	33.62	33.59	33.46	33.51	33.27	33.56
35	33.50	33.65	33.59	33.61	33.49	33.51	33.27	33.59
36	33.50	33.67	33.60	33.66	33.53	33.55	33.29	33.59
37	33.51	33.68	33.58	33.68	33.55	33.60	33.34	33.62
38	33.55	33.70	33.62	33.69	33.58	33.68	33.41	33.66
39	33.61	33.74	33.69	33.71	33.59	33.68	33.48	33.66
40	33.65	33.76	33.76	33.75	33.65	33.63	33.53	33.67
41	33.67	33.80	33.79	33.78	33.65	33.64	33.59	33.71
42	33.75	33.83	33.79	33.80	33.65	33.66	33.63	33.74
43	33.81	33.88	33.83	33.83	33.65	33.69	33.72	33.76
44	33.80	33.90	33.92	33.86	33.68	33.70	33.86	33.80
45	33.79	33.89	33.95	33.87	33.72	33.69	33.96	33.81
46	33.84	33.91	33.98	33.89	33.73	33.76	34.05	33.85
47	33.89	33.93	34.03	33.90	33.76	33.83	34.15	33.87
48	33.91	33.93	34.00	33.91	33.76	33.83	34.23	33.87
49	33.93	33.95	33.98	33.90	33.77	33.79	34.29	33.86
50	33.94	33.95	33.98	33.89	33.83	33.77	34.35	33.87
51	33.95	33.92	33.95	33.91	33.86	33.78	34.36	33.91
52	33.97	33.93	33.98	33.92	33.84	33.82	34.35	33.91
53	33.99	34.02	34.01	33.96	33.90	33.84	34.29	33.88
54	34.01	34.10	34.04	34.03	33.94	33.84	34.22	33.87
55	34.06	34.17	34.08	34.10	34.01	33.85	34.18	33.91
56	34.21	34.21	34.15	34.15	34.02	33.90	34.11	33.98
57	34.23	34.22	34.17	34.16	34.02	33.94	34.07	34.00
58	34.19	34.23	34.16	34.18	34.01	33.95	34.03	34.00
59	34.21	34.23	34.19	34.22	33.99	33.99	34.02	34.01
60	34.17	34.24	34.22	34.24	34.00	34.02	34.01	34.03

Table E.12: Volunteer 6 Left side. Temperature values (°C) at different time periods of NPWT dressing application (with and without suction at -400 mmHg).

Distance from foam (mm)	Time Periods							
	Pre-NPWT	Dressing on					Dressing off	
		Suction off	Suction on		Suction off		5 min	5 min
	Baseline	Baseline	1 min	5 min	1 min	5 min		
1	32.16	32.72	32.89	31.34	30.75	30.13	33.98	33.85
2	32.25	33.11	33.14	32.28	31.70	31.03	33.85	33.77
3	32.27	33.28	33.09	32.79	31.91	31.90	33.73	33.72
4	32.25	33.30	33.18	32.54	32.08	32.14	33.64	33.70
5	32.29	33.42	33.15	32.54	32.14	32.34	33.50	33.65
6	32.60	33.51	33.02	32.40	32.12	32.43	33.40	33.62
7	32.49	33.57	33.14	32.36	32.52	32.59	33.35	33.61
8	32.47	33.47	33.20	32.36	32.64	32.90	33.34	33.62
9	32.39	33.48	33.21	32.68	33.00	32.97	33.35	33.59
10	32.43	33.49	33.24	32.79	33.21	33.33	33.33	33.54
11	32.54	33.54	33.25	32.90	33.22	33.42	33.32	33.51
12	32.63	33.54	33.27	32.98	33.27	33.49	33.29	33.50
13	32.69	33.55	33.31	33.01	33.10	33.26	33.29	33.50
14	32.70	33.57	33.33	33.02	33.15	33.27	33.31	33.52
15	32.73	33.52	33.36	33.12	33.21	33.31	33.33	33.52
16	32.76	33.48	33.32	33.18	33.24	33.35	33.30	33.55
17	32.78	33.44	33.28	33.22	33.26	33.41	33.33	33.59
18	32.83	33.38	33.29	33.29	33.26	33.43	33.38	33.60
19	32.87	33.39	33.38	33.29	33.18	33.38	33.39	33.61
20	32.90	33.51	33.49	33.02	33.19	33.12	33.42	33.58
21	32.92	33.57	33.50	33.27	33.31	33.30	33.48	33.54
22	32.90	33.58	33.47	33.40	33.42	33.43	33.47	33.54
23	32.94	33.58	33.44	33.49	33.44	33.50	33.47	33.52
24	33.03	33.54	33.44	33.49	33.42	33.49	33.49	33.51
25	33.07	33.52	33.47	33.49	33.38	33.49	33.48	33.49
26	33.11	33.56	33.49	33.47	33.37	33.50	33.49	33.50
27	33.11	33.59	33.48	33.45	33.40	33.45	33.49	33.50
28	33.08	33.54	33.43	33.44	33.40	33.42	33.44	33.47
29	33.06	33.49	33.43	33.44	33.35	33.40	33.40	33.45
30	33.07	33.47	33.43	33.43	33.36	33.40	33.42	33.45
31	33.10	33.45	33.38	33.39	33.40	33.41	33.43	33.44
32	33.12	33.47	33.34	33.35	33.37	33.43	33.43	33.41
33	33.12	33.51	33.38	33.37	33.33	33.43	33.45	33.43

Table E.12 continued: Volunteer 6 Left side. Temperature values (°C) at different time periods of NPWT dressing application (with and without suction at -400 mmHg).

Distance from foam (mm)	Time Periods							
	Pre-NPWT	Dressing on					Dressing off	
		Suction off	Suction on		Suction off		5 min	5 min
	Baseline	Baseline	1 min	5 min	1 min	5 min		
34	33.12	33.50	33.41	33.37	33.37	33.39	33.45	33.46
35	33.13	33.51	33.43	33.37	33.48	33.42	33.38	33.49
36	33.14	33.54	33.43	33.43	33.50	33.47	33.32	33.51
37	33.16	33.52	33.46	33.48	33.44	33.48	33.28	33.47
38	33.16	33.48	33.48	33.50	33.41	33.47	33.34	33.47
39	33.17	33.50	33.50	33.54	33.44	33.48	33.43	33.49
40	33.13	33.49	33.50	33.56	33.45	33.50	33.45	33.47
41	33.02	33.48	33.48	33.56	33.43	33.52	33.49	33.47
42	32.98	33.50	33.45	33.54	33.43	33.51	33.50	33.47
43	32.92	33.47	33.40	33.49	33.43	33.49	33.47	33.47
44	32.90	33.44	33.38	33.40	33.41	33.45	33.45	33.45
45	32.95	33.46	33.38	33.50	33.42	33.45	33.45	33.40
46	32.99	33.44	33.41	33.49	33.43	33.43	33.43	33.36
47	33.01	33.45	33.41	33.45	33.43	33.44	33.40	33.37
48	33.03	33.47	33.39	33.45	33.42	33.45	33.42	33.40
49	33.05	33.49	33.39	33.46	33.40	33.46	33.43	33.38
50	33.05	33.45	33.39	33.47	33.39	33.47	33.40	33.35
51	33.09	33.40	33.40	33.44	33.40	33.46	33.40	33.40
52	33.14	33.41	33.43	33.43	33.42	33.47	33.43	33.43
53	33.14	33.45	33.45	33.41	33.44	33.47	33.43	33.43
54	33.13	33.51	33.45	33.42	33.44	33.50	33.43	33.43
55	33.10	33.51	33.45	33.45	33.49	33.50	33.39	33.43
56	33.05	33.47	33.44	33.47	33.48	33.50	33.36	33.46
57	33.05	33.45	33.45	33.47	33.41	33.52	33.38	33.48
58	33.12	33.48	33.46	33.47	33.42	33.55	33.41	33.50
59	33.11	33.50	33.46	33.48	33.45	33.55	33.41	33.50
60	33.08	33.49	33.46	33.49	33.43	33.57	33.43	33.49

Table E.13: Volunteer 6 Right side. Temperature values (°C) at different time periods of NPWT dressing application (with and without suction at -400 mmHg).

Distance from foam (mm)	Time Periods							
	Pre-NPWT	Dressing on					Dressing off	
		Suction off	Suction on		Suction off		5 min	5 min
	Baseline	Baseline	1 min	5 min	1 min	5 min		
1	32.76	32.52	32.77	32.42	31.37	31.10	33.82	33.68
2	32.82	32.63	32.72	32.86	32.47	32.47	33.69	33.64
3	32.74	32.75	32.75	32.86	32.65	32.90	33.61	33.64
4	32.72	32.76	32.78	32.88	32.94	33.02	33.52	33.62
5	32.70	32.76	32.78	32.85	33.01	33.10	33.41	33.56
6	32.74	32.79	32.78	32.87	33.05	33.15	33.43	33.56
7	32.76	32.82	32.81	32.92	33.05	33.17	33.52	33.57
8	32.82	32.90	32.84	32.92	33.01	33.12	33.50	33.56
9	32.88	32.99	32.88	32.94	33.03	33.14	33.44	33.56
10	32.90	33.01	32.90	32.98	33.09	33.18	33.41	33.57
11	32.91	33.01	32.91	33.01	33.14	33.23	33.36	33.54
12	32.94	33.03	32.93	33.03	33.14	33.27	33.34	33.52
13	32.98	33.00	32.97	33.05	33.07	33.29	33.35	33.50
14	32.99	32.97	32.95	33.01	33.05	33.22	33.33	33.47
15	32.98	32.99	32.93	32.99	33.05	33.20	33.33	33.45
16	32.96	32.97	32.94	33.02	33.02	33.17	33.33	33.42
17	32.94	32.94	32.94	33.05	33.02	33.15	33.30	33.35
18	32.98	32.93	32.95	33.05	33.05	33.14	33.28	33.27
19	33.03	32.92	32.99	33.05	33.06	33.13	33.25	33.26
20	33.01	32.91	32.98	33.04	33.03	33.10	33.22	33.28
21	32.96	32.94	32.95	32.99	33.01	33.05	33.21	33.25
22	32.92	33.02	33.01	32.97	33.03	33.01	33.22	33.26
23	32.97	33.03	33.09	33.00	33.06	33.01	33.21	33.29
24	32.97	33.01	33.13	33.07	33.14	33.10	33.20	33.32
25	32.93	32.99	33.13	33.14	33.23	33.15	33.19	33.31
26	32.92	32.99	33.11	33.15	33.24	33.14	33.15	33.25
27	32.92	33.01	33.11	33.21	33.19	33.16	33.16	33.26
28	32.90	33.04	33.09	33.26	33.21	33.19	33.23	33.33
29	32.89	32.76	33.13	33.28	33.23	33.24	33.28	33.36
30	32.87	33.02	33.14	33.26	33.24	33.29	33.26	33.34
31	32.84	33.03	33.12	33.26	33.24	33.29	33.26	33.36
32	32.80	33.05	33.12	33.26	33.24	33.26	33.28	33.40
33	32.78	33.09	33.11	33.25	33.23	33.24	33.28	33.40

Table E.13 continued: Volunteer 6 Right side. Temperature values (°C) at different time periods of NPWT dressing application (with and without suction at -400 mmHg).

Distance from foam (mm)	Time Periods							
	Pre-NPWT	Dressing on					Dressing off	
		Suction off	Suction on		Suction off		5 min	5 min
	Baseline	Baseline	1 min	5 min	1 min	5 min		
34	32.86	33.12	33.13	33.17	33.25	33.25	33.29	33.35
35	32.90	33.13	33.18	33.22	33.30	33.25	33.27	33.33
36	32.93	33.16	33.20	33.24	33.30	33.21	33.24	33.38
37	32.99	33.16	33.20	33.22	33.28	33.21	33.25	33.41
38	33.05	33.17	33.20	33.23	33.27	33.24	33.22	33.42
39	33.11	33.14	33.22	33.24	33.28	33.25	33.26	33.40
40	33.12	33.10	33.22	33.24	33.27	33.25	33.28	33.39
41	33.10	33.08	33.22	33.26	33.30	33.26	33.31	33.39
42	33.19	33.11	33.27	33.29	33.30	33.30	33.29	33.38
43	33.21	33.16	33.27	33.32	33.31	33.32	33.32	33.44
44	33.16	33.15	33.22	33.30	33.33	33.30	33.31	33.49
45	33.12	33.12	33.23	33.25	33.33	33.25	33.28	33.50
46	33.19	33.18	33.32	33.28	33.33	33.31	33.27	33.56
47	33.24	33.26	33.32	33.35	33.26	33.37	33.28	33.59
48	33.26	33.27	33.26	33.38	33.36	33.38	33.27	33.61
49	33.26	33.30	33.31	33.41	33.37	33.40	33.28	33.62
50	33.27	33.32	33.38	33.43	33.43	33.40	33.35	33.65
51	33.30	33.36	33.41	33.43	33.49	33.40	33.40	33.72
52	33.30	33.40	33.43	33.47	33.55	33.45	33.40	33.73
53	33.27	33.42	33.47	33.49	33.57	33.49	33.44	33.75
54	33.29	33.44	33.49	33.50	33.54	33.52	33.53	33.78
55	33.34	33.41	33.49	33.52	33.54	33.59	33.57	33.77
56	33.35	33.41	33.48	33.52	33.54	33.63	33.59	33.73
57	33.31	33.37	33.48	33.54	33.56	33.63	33.62	33.73
58	33.29	33.39	33.51	33.57	33.59	33.61	33.63	33.78
59	33.24	33.42	33.52	33.61	33.59	33.63	33.67	33.80
60	33.21	33.46	33.53	33.62	33.59	33.68	33.70	33.78

Appendix F.
Experiment data: Laser Doppler study

F1. Laser Doppler study

F1.1. First volunteer

Table F.1: Laser Doppler perfusion units during various sequences of NPWT at -125 mmHg and manual pressure (1.8 kg) in different anatomical regions.

Region	Sequence order	Perfusion Units			
		Mean	SD	Min	Max
Back	Resting	7.9	1.2	5.1	12.6
	NPWT	7.3	2	4.5	53.8
	Weight	7	1.2	4.3	12.8
Back	Resting	12.2	1.8	8.4	26.2
	Weight	8.7	2.2	5.2	21.2
	NPWT	11.2	2.5	7.3	65.1
Chest	Resting	10.4	2.6	5.6	53.3
	NPWT	9.6	4.6	5.2	114
	Weight	10.2	3.9	4.5	53.7
Chest	Resting	10.5	2.4	5.7	22.5
	Weight	20.2	3.4	13.3	33.7
	NPWT	13.7	8.7	6.7	225.7
Arm	Resting	31	5.5	17.3	74.1
	NPWT	37.3	5.5	22.7	58.8
	Weight	34	5.1	19.8	51.6
Leg	Resting	5.4	0.9	2.8	10.9
	NPWT	6.7	1.4	3.9	27.3
	Weight	7.6	1.1	4.1	13.1

F.1.2. Second volunteer

Table F.2: Laser Doppler perfusion units during various sequences of NPWT at -125 mmHg and manual pressure (1.8 kg) in different anatomical regions.

Region	Sequence order	Perfusion Units			
		Mean	SD	Min	Max
Back	Resting	11.2	1.7	7	20.1
	NPWT	11.9	2.1	6.1	24.7
	Weight	11.3	1.8	6.1	28.2
Back	Resting	10.8	1.9	6.7	41.7
	Weight	9.5	1.4	5.9	19.3
	NPWT	10.8	1.8	6.2	23.9
Chest	Resting	8.4	1.3	5.6	19
	NPWT	9.1	1.9	5.5	45.7
	Weight	15.9	4.2	6.6	76.1
Chest	Resting	26.8	6.3	13.7	69.7
	Weight	39.4	8.1	23.6	116.7
	NPWT	12.8	10.2	5	204.9
Arm	Resting	12	1.7	8.1	24.8
	NPWT	10.4	1.7	6.4	23.5
	Weight	9.8	3.1	5.5	48.4
Leg	Resting	11.2	2.9	5.1	30
	NPWT	11.1	2.4	6.3	24.5