LUND UNIVERSITY

Negative Pressure Wound Therapy. Therapy Settings and Biological Effects in Peripheral Wounds

Borgquist, Ola

2013

Negative Pressure Wound Therapy

Therapy Settings and Biological Effects

in Peripheral Wounds

Ola Borgquist, M.D.

When told, "I'm too busy treating patients to do research," I answer:

When you treat a patient, you have treated 1 patient.

When you do research, you have treated 10,000 patients.

Robert Riffenburgh, author of "Statistics in Medicine"

Contents

Abstract

Negative pressure wound therapy (NPWT) promotes wound healing through several mechanisms, e.g., altered periwound blood flow, mechanical deformation of the wound edge tissue, and drainage of excess fluid and debris. The general aim of this thesis was to study the impact of different levels of negative pressure, different wound filling materials (foam or gauze), and different ways of applying the negative pressure (continuously, intermittently or variably) on the biological effects of NPWT in peripheral wounds. The intention was to provide a scientific basis for the choice of these parameters in order to be able to optimize the healing of NPWT-treated wounds in the future.

Studies were carried out on peripheral wounds created on the backs of pigs. The effect of NPWT on periwound blood flow was investigated using invasive and transcutaneous laser Doppler flowmetry, as well as thermodiffusion. Blood flow was found to decrease 0.5 cm laterally from the wound edge and increase 2.5 cm from the wound edge; a transition zone being seen 1 cm from the wound edge. Blood flow changed gradually with increasing levels of negative pressure, reaching half maximal effect at approximately –45 mmHg and maximum effect at about –80 mmHg. The blood flow response was found to depend on the measurement technique. Applying intermittent and variable pressure resulted in concomitant increases and decreases in periwound blood flow. The combination of hypo- and hyperperfusion may be beneficial in the process of wound healing.

The mechanical effects of NPWT were studied with regards to macro- and microdeformation. It was found that the degree of macrodeformation, i.e., wound contraction, increased gradually with increasing negative pressure level, reaching half maximal effect at about –45 mmHg and near-maximal effect at –75 mmHg. The degree of wound contraction was the same regardless of whether foam or gauze was used as wound filler. The effects of NPWT on microdeformation, i.e., the microscopic interaction between the wound filler and the newly formed granulation tissue, were examined histologically using stained sections of the wound bed. Both foam- and gauze-based NPWT were shown to induce microdeformation of the wound bed tissue.

The effect of NPWT on fluid evacuation from the wound cavity was measured gravimetrically. The amount of evacuated fluid increased gradually with increasing level of negative pressure, reaching a near-maximum at –125 mmHg. It may thus be

beneficial to treat wounds containing large volumes of exudate with a high negative pressure initially (e.g., –125 mmHg), and then reduce the pressure to a level more appropriate for the wound edge tissue.

In conclusion, the biological effects of NPWT were influenced by the negative pressure level, the wound filling material and the way in which NPWT was applied (continuously, intermittently or variably). Hopefully, the results of these studies may provide a scientific basis for the choice of NPWT parameters in the treatment of wounds. Further clinical studies are needed to corroborate our findings before recommendations can be made regarding the NPWT settings for treatment of different wound types and tissues in order to improve wound healing.

Populärvetenskaplig sammanfattning

Summary in Swedish

Undertrycksbehandling av sår (*negative pressure wound therapy*, NPWT) har förekommit under lång tid i olika utföranden. Sedan slutet av 1990-talet har användningen av tekniken ökat kraftigt inom flera kliniska discipliner eftersom effekten på sårläkning visat sig vara mycket god för många olika sårtyper. Trots omfattande forskning saknas det fortfarande kunskaper om hur olika inställningar av behandlingen påverkar såret och den omkringliggande vävnaden. Fördjupade kunskaper om detta kan förhoppningsvis leda till förbättrad sårläkning och minskad risk för komplikationer.

I princip finns det idag tre variabler som kan användas för att styra behandlingen: undertrycksnivå, sårfyllnadsmaterial och applikationssätt. Traditionellt används en förhållandevis hög undertrycksnivå, −125 mmHg. Som sårfyllnadsmaterial används polyuretanskum eller gasväv. Vad det gäller applikationssätt används oftast kontinuerlig behandling, vilket innebär att undertrycksnivån hålls konstant (t ex −75 mmHg). Om trycknivån i stället alternerar mellan atmosfärstryck och, exempelvis, −75 mmHg benämns detta "intermittent behandling". Variabel undertrycksbehandling liknar den intermittenta behandlingen, men trycket förblir hela tiden negativt (man alternerar t ex mellan −40 och −75 mmHg) och övergången mellan undertrycksnivåerna sker gradvis för att minska problemen med smärta hos patienterna. Cyklerna är vanligtvis sju minuter långa: fem minuter med högt undertryck och två minuter med lågt undertryck eller atmosfärstryck.

Syftet med studierna har varit att analysera de olika behandlingsinställningarnas påverkan på väsentliga läkningsfaktorer (t ex blodflödesförändringar, sårkontraktion och vätskedränage) i sår och sårkant. Om man känner till effekten på sårvävnaden kan man förhoppningsvis anpassa behandlingen för att optimera sårläkningen. Det är mycket svårt att göra randomiserade, kontrollerade studier på en heterogen patientgrupp med sår av varierande etiologi. Vi har därför använt sår på grisar för våra experiment. Grisens hud liknar människans.

I studie I visar vi att blodflödet i sårkanten ändras beroende på vilken undertrycksnivå som används och avståndet till sårkanten. Nära sårkanten (0,5 cm bort) minskar

blodflödet när NPWT appliceras. Detta beror förmodligen på att vävnaden pressas ihop under behandlingen. Längre bort (2,5 cm från sårkanten) ökar genomblödningen. Mekanismen för detta är okänd. De beskrivna blodflödesförändringarna ses redan vid låga undertrycksnivåer och ökar allteftersom undertrycket ökar. Vid ca –80 mmHg nås en platåfas. Därefter sker inga ytterligare blodflödesändringar trots ökande undertrycksnivåer. Kombinationen av ökat och minskat blodflöde i vävnaden invid såret är sannolikt betydelsefull för sårläkningen. I studie II visar vi att valet av mätteknik påverkar blodflödesändringarna. Icke-invasiva mätningar med transkutan laser Doppler-teknik förefaller ge resultat som inte helt överensstämmer med invasiva mätningar med laser Doppler och termodiffusion. I studie III visas att intermittent och variabel undertrycksbehandling ger likartade blodflödeseffekter i sårkanten.

Enligt resultaten från studie IV förefaller graden av mekanisk påverkan på sårkanten (i form av makro- och mikrodeformation) vara densamma oavsett om skum eller gasväv används som sårfyllnadsmaterial. Sårkontraktionen kvarstår när behandlingen avslutats, 72 timmar efter påbörjandet. I studie V visar vi att ca –75 mmHg ger maximal sårkontraktion. En högre undertrycksnivå (–125 mmHg) kan vara att föredra när det finns stora mängder vätska som behöver evakueras från såret.

Även om undertrycksbehandling i dess nuvarande form uppvisar goda resultat, finns det utrymme att anpassa behandlingen så att sårläkningen förbättras och antalet komplikationer minskas. Med nyvunnen kunskap om hur behandlingsinställningarna påverkar såret och omkringliggande vävnad ges nu underlag för hur man kan variera behandlingen så att varje patient erbjuds bästa möjliga sårläkning. Exempelvis finns det sannolikt inte *en* optimal undertrycksnivå, utan snarare ett spann mellan –25 och –125 mmHg där man kan uppnå olika effekter i sårvävnaden. I framtiden kommer förhoppningsvis sårfyllnadsmaterialet, undertrycksnivån och applikationssättet av NPWT att väljas utifrån sårets art, lokalisation och sammansättning. Resultaten i denna avhandling kommer från djurexperimentella studier och ger en grundläggande förståelse för undertrycksbehandlingens biologiska effekter i såret. Ytterligare studier behövs innan kliniska rekommendationer kan utfärdas.

Papers included in this thesis

This thesis is based on the following five papers, which are referred to in the text by their Roman numerals:

I   **Borgquist O**, Ingemansson R, Malmsjö M
    Wound edge microvascular blood flow during negative pressure wound therapy: examining the effects of pressures from -10 to -175 mmHg
    Plast Reconstr Surg 2010 Feb;125(2):502–9

II   **Borgquist O**, Anesäter E, Hedström E, Lee CK, Ingemansson R, Malmsjö M
    Measurements of wound edge microvascular blood flow during negative pressure wound therapy using thermodiffusion and transcutaneous and invasive laser Doppler flowmetry
    Wound Rep Regen 2011 Nov;19(6):727–33

III **Borgquist O**, Ingemansson R, Malmsjö M
    The effect of intermittent and variable negative pressure wound therapy on wound edge microvascular blood flow
    Ostomy Wound Management 2010;56(3):60–7

IV   **Borgquist O***, Gustafsson L*, Ingemansson R, Malmsjö M
    Micro- and macromechanical effects on the wound bed by negative pressure wound therapy using gauze and foam
    Ann Plast Surg 2010 Jun;64(6):789–93

V   **Borgquist O**, Ingemansson R, Malmsjö M
    The influence of low and high pressure levels during negative pressure wound therapy on wound contraction and fluid evacuation
    Plast Reconstr Surg 2011 Feb;127(2):551–9

* These authors contributed equally.

Other relevant publications

Original articles

1. **Borgquist O**, Gustafsson L, Ingemansson R, Malmsjö M
   Tissue ingrowth into foam but not into gauze during negative pressure wound therapy
   Wounds 2009;21(11):302–9

2. Anesäter E, **Borgquist O**, Hedström E, Waga J, Ingemansson R, Malmsjö M
   The influence of different sizes and types of wound fillers on wound contraction and tissue pressure during negative pressure wound therapy
   Int Wound J 2011 Aug;8(4):336–42

3. Anesäter E, Roupé KM, Robertsson P, **Borgquist O**, Torbrand C, Ingemansson R, Lindstedt S, Malmsjö M
   The influence on wound contraction and fluid evacuation of a rigid disc inserted to protect exposed organs during negative pressure wound therapy
   Int Wound J 2011 Aug;8(4):393–9

4. Anesäter E, **Borgquist O**, Torbrand C, Roupé KM, Ingemansson R, Lindstedt S, Malmsjö M
   The use of a rigid disc to protect exposed structures in wounds treated with negative pressure wound therapy – effects on wound bed pressure and microvascular blood flow
   Wound Repair Regen 2012 Jul;20(4):611–6

5. Anesäter E, **Borgquist O**, Torbrand C, Roupé KM, Ingemansson R, Lindstedt S, Malmsjö M
   A rigid disc for protection of exposed blood vessels during negative pressure wound therapy
   Surg Innov 2013 Feb;20(1):74–80

Review articles

1. Malmsjö M, **Borgquist O**
   NPWT settings and dressing choices made easy
   Wounds International 2010;1(3)

2. **Borgquist O**, Ingemansson R, Malmsjö M
   Individualizing the use of negative pressure wound therapy for optimal wound healing: a focused review of the literature
   Ostomy Wound Management 2011 Apr;57(4):44–54

3. **Borgquist O**, Ingemansson R, Lindstedt S, Malmsjö M
   Undertrycksbehandling av sår – kunskap om verkningsmekanismer och komplikationer ger nya möjligheter
   [Negative pressure wound therapy. Knowledge of mechanisms and complications provides new opportunities]
   Läkartidningen 2011;46(108):2372–5. In Swedish

Introduction

The increasing number of chronic and complex wounds poses a growing medical challenge with a high socioeconomic impact and a significant burden to the individual patient. The management of chronic wounds places a considerable burden on health services as it requires considerable manpower, frequent specialist consultation, and adjunct therapies. Generally, figures presented on the cost of wound care only reflect the direct medical costs, and do not include the pain, frustration, economic loss or impaired quality of life of the patients and their families, or the burden on the health care system or society in general. Fortunately, several important changes in the principles of wound care have taken place in recent decades. Based on these new insights, a wide range of wound care techniques has been introduced. Negative pressure wound therapy (NPWT) is one of these.

Wound healing

Wound healing is a dynamic, interactive process involving soluble mediators, blood cells, extracellular matrix components, resident cells (keratinocytes, fibroblasts, endothelial cells, nerve cells), and infiltrating leukocyte subtypes in a complex and highly orchestrated sequence of cellular and biochemical changes. Some wounds heal naturally, and these are referred to as acute wounds. Although there is no consensus on the definition of acute wounds, they are normally defined as wounds present for only a short period of time (<4 weeks to a maximum of 6 weeks) before intervention and healing [1]. Chronic wounds, on the other hand, are defined as those present for more than 4 to 6 weeks prior to intervention, without any tendency to normal wound healing. These wounds either require a long time to heal, do not heal completely, or recur frequently [2]. Examples of common chronic wounds are pressure ulcers, ischemic ulcers, venous leg ulcers, and diabetic foot ulcers.

Several stages are involved in the normal healing of an open wound. Firstly, either the edges of the wound must be brought together to form a seal ("healing by primary intention"), or granulation tissue must form before closure can take place ("healing by secondary intention"). Secondly, the environment around the wound must be moist, as new epidermal cells can only travel across moist surfaces. Thirdly, bacterial infection must be prevented by maintaining aseptic conditions. Fourthly, excess fluid should be removed from the wound site, while maintaining an appropriate level of

moisture. Finally, the cause of the wound should be eliminated or, if this is not possible, minimized (as may be the case with pressure ulcers in bedridden patients).

Successful wound healing has four phases that overlap in time: hemostasis (first 24 hours), inflammation (~0–48 h), tissue formation (~48 h–6 weeks), and tissue remodeling (~3 weeks–2 years) (Figure 1) [3]. The length of these phases may vary as a result of infection, malnutrition, or other exogenous factors.
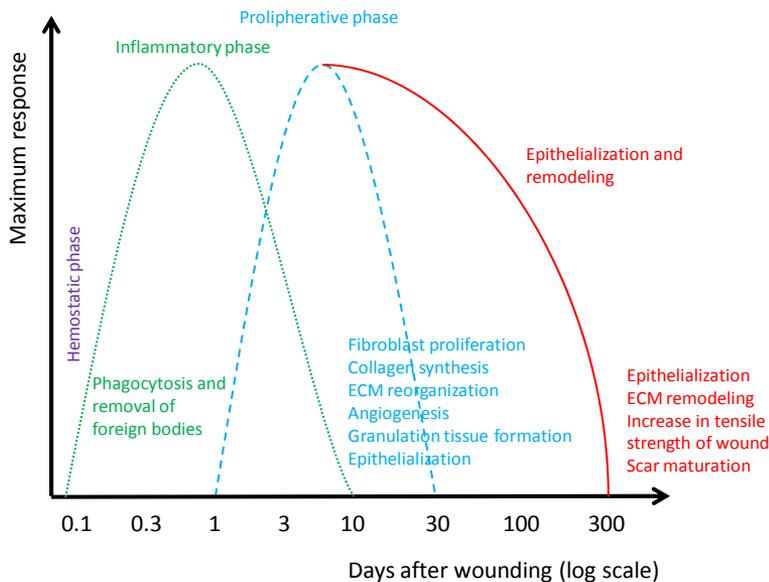
Figure 1. The temporal aspects of the four phases of wound healing (hemostasis, inflammation, tissue formation, and tissue remodeling). ECM = extracellular matrix. Adapted from [4].

The initial inflammatory responses to injury provide the necessary framework for the production of a new functional barrier. Physiologically, the inflammatory phase begins at the time of injury and lasts between 4 and 6 days. This phase begins with hemostasis, involving the extravasation of leukocytes, leading to inflammation, characterized by erythema and edema of the wound edges. Injury also disrupts the normal skin barrier, and wound healing thus depends on the ability to clear foreign material and resist infection.

The proliferative phase involves elementary processes such as neovascularization, the formation of granulation tissue and the extracellular matrix, and the creation of a permeable barrier (reepithelialization). Fibroblasts proteolytically degrade the provisional matrix so as to permit its replacement with an extracellular matrix composed primarily of fibrillar collagens and proteoglycans. Fibroblast proliferation is oxygen-dependent and the cells can only survive and perform the functions necessary in the wound when sufficient oxygen is available [5]. The moving band of phagocytosing macrophages typically leads the fibroblast front into the wound [6]. This phase has a rapid onset, but takes considerably longer to reach its peak, usually 2–3 weeks after the injury.

The final phase, remodeling, is an essential component of tissue repair, bringing about changes in the composition of the extracellular matrix. This results in an organized and functional scar that has properties similar to the parent tissue. This phase begins about 2 to 3 weeks after the injury and can last up to 2 years.

Treatment of wounds

The aim in the treatment of any type of wound is to achieve normal and timely healing. Fundamental management principles for optimal healing outcomes are given in Table 1 below.

Table 1. Management principles for optimal healing outcomes. Adapted from [7].

Optimization of the patient's health status	- Nutritional support - Adequate hydration - Glycemic control - Optimal control of comorbidity - Smoking cessation - Moderation of alcohol intake
Treatment of the underlying cause of the wound	- Good blood flow and tissue perfusion - Compression therapy for edema and venous insufficiency in the absence of arterial disease - Optimization using offloading devices for the management of: - Diabetic foot ulcers and pressure ulcers - Pressure redistribution - Reduction of friction/shear stress - Surgical intervention to correct physical deformities
Optimization of the wound bed and local wound environment	- Wound debridement - Maintenance of moisture balance - Maintenance of normothermic wound environment - Treatment to reduce bacterial burden or deep infection

The large number of factors that may affect wound healing, such as tissue oxygen tension, wound hydration, temperature, and mechanical stress, together with the high degree of variation in wound characteristics and patient status may make it difficult to evaluate the effects of treatment. In some cases, wound healing techniques are not expected to result in complete wound closure. Rather, the treatment may be intended to advance the wound to a stage where healing is possible.

Negative pressure wound therapy

NPWT involves the controlled application of subatmospheric pressure to the wound bed through a wound filler (foam or gauze) placed in the wound. The wound is then sealed with an adhesive drape that allows pressure to be applied and helps provide a moist environment that supports wound healing. The wound filler and drape protect the wound bed from bacteria and other contaminants, as well as other bodily fluids, and reduce the risk of friction or shear, enhancing the body's ability to heal [8]. The hypobaric pressure removes exudate and promotes healing in various ways.

There are many documented cases of NPWT in wound healing throughout history. It was first used successfully by Raffl in the early 1950s to manage exudate and accelerate wound healing after radical mastectomies [9]. A number of NPWT systems were then tried in the 1980s, with both USA and Russia in the forefront of the technology. Miller *et al.* [10] quote a series of articles describing the treatment of wounds using negative pressure, the "Kremlin papers" [11], that was published in the Russian literature in the 1980s. Negative pressure (–75 to –80 mmHg) was used in combination with aggressive debridement to significantly reduce bacterial counts in purulent wounds [12]. In 1989, Chariker *et al.* [13] discussed their experience utilizing NPWT in patients with incisional or cutaneous fistulae complicating ventral abdominal wounds. Moist gauze was placed over the wound surface and a flat drain placed over the gauze and covered with an occlusive dressing. The drain was connected to an existing vacuum line such as a standard hospital wall suction source with continuous pressure set at approximately –60 to –80 mmHg. This method later became known as the Chariker-Jeter technique.

In the early 1990s, the first investigative studies into NPWT using foam as a wound contact layer were carried out by Fleischmann *et al.* [14-16]. Argenta and Morykwas also carried out work on the use of foam contact layers in both patients and animals, and found that the use of NPWT with a pressure setting of –125 mmHg was likely to reduce healing time, reduce local edema and manage wound exudate [17]. Their work led to the development of the first commercialized system, the V.A.C.® Therapy System (KCI, San Antonio, TX). Until 2003, this was the only commercially available NPWT system. However, the Chariker-Jeter approach resurfaced in 2003 after having remained largely unknown due to the publication of the method in a non peer-reviewed journal, and the lack of follow-up articles by its authors. There are now

currently two forms of NPWT systems in use in wound management, with the main difference between them being in the type of dressing used to fill the wound (foam or gauze).

NPWT has become an integral part of modern wound care, and is used routinely in hospitals throughout the world. It is estimated that 300 million acute wounds are treated globally each year [18]. NPWT has successfully been used for the treatment of acute, chronic and complex wounds [19-25]. There is a substantial body of clinical and economic evidence supporting the efficacy of NPWT in wound management, including early discharge and faster healing, fewer readmissions, and better patient quality of life [26-30].

NPWT cannot replace surgical procedures, but may allow a wound to progress to the point at which a less invasive procedure is possible [31]. It is being increasingly recognized that NPWT can be used to achieve a variety of treatment goals, which vary according to the patient and the characteristics of the wound [32]. Some of the common goals of the therapy are:

- to promote rapid reduction in wound volume,
- to promote the growth of granulation tissue and contraction of wound edges [23],
- to manage exudate [27],
- to prepare the wound bed for transition to another treatment modality,
- to decrease the frequency of dressing change [26],
- to prevent deterioration of the wound,
- to minimize contamination and wound odor by providing a temporary barrier,
- to decrease morbidity and mortality,
- to improve quality of life, and
- to decrease the length of hospital stay.

NPWT is contraindicated when there is necrotic tissue with eschar, inadequate debridement, or in the presence of untreated osteomyelitis or sepsis in the wound area. It is also contraindicated in cases of untreated coagulopathy, an exposed vital organ, or malignancy in the wound, or if the patient is allergic to any vital component of the dressing, drape, or NPWT device.

Mechanisms of action of NPWT

The physiological, cellular and molecular mechanisms by which NPWT accelerates wound healing are now gradually starting to be understood [33-40]. Basically, NPWT can be regarded as a method that combines the advantages of closed- and open-wound management [41]. Closed-wound treatment, involving approximation of skin edges, may protect the wound from bacterial invasion, and can also be expected to maintain moisture, pH, gaseous concentrations, and temperature at

optimal levels for healing. Open treatment, however, allows for the release of toxic products, and enables regular monitoring and assessment of a complex wound.

There appears to be no single mechanism of action that can explain the beneficial effect of NPWT on wounds. Rather, multiple mechanisms have been identified (Figure 2), which together contribute to the observed clinical effects. Preclinical and clinical studies have shown that NPWT provides a moist wound healing environment [42], drains exudate [17, 43, 44], reduces tissue edema [17, 43, 45], contracts wound edges [17, 43, 44, 46-49], mechanically stimulates the wound bed [50, 51], alters blood flow in and around the wound edges [17, 36, 37, 52], and stimulates angiogenesis [17, 53-56] and the formation of granulation tissue [17]. It is thus believed that the mechanisms of action of NPWT have largely been identified. However, little is known about the influence of different NPWT settings on these biological effects in the wound. The work presented in this thesis deals with the impact of NPWT and its settings on blood flow, mechanical deformation and wound fluid removal. These areas are further discussed below.
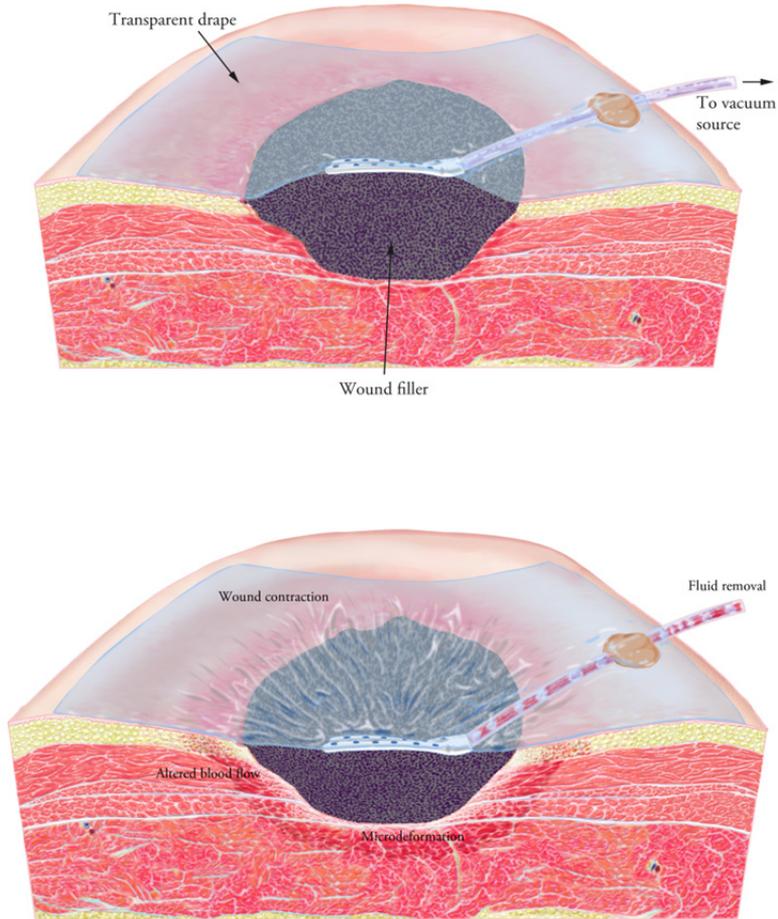
Figure 2. Cross-section of a peripheral wound treated with foam-based NPWT. The top image shows the wound before application of negative pressure, while the lower image shows the wound after application of NPWT. When applying NPWT to a wound, the wound is first debrided and then filled with a material that will deliver negative pressure to the wound bed (in this case, foam). The wound is sealed with an adhesive plastic drape, and a drain is connected to the vacuum pump. The pressure applied by the vacuum pump is propagated through the wound filler to the wound bed. Some of the biological effects of the therapy are illustrated in the lower image. There is a decrease in blood flow very close to the wound edge (white), whereas increased blood flow is seen further away (dark red). The wound is contracted and fluid is evacuated through the drainage tube. Illustration by Bo Veisland.

Blood flow

NPWT is known to affect periwound blood flow. Morykwas *et al.* [17] were the first to investigate changes in blood flow due to NPWT. In their rather limited study, blood flow was measured with laser Doppler flowmetry in a porcine wound model, and it was shown that the periwound blood flow increased upon the application of NPWT, peaking at a pressure of –125 mmHg [17]. This paper led to –125 mmHg being adopted as the universally recommended pressure in the clinical setting. However, as clinical experience with NPWT grew bigger, the use of –125 mmHg was questioned as it seemed to cause patient pain and wound ischemia [57, 58].

Morykwas *et al.* investigated the effects of both continuous and intermittent therapy [17]. After five to seven minutes of continuous therapy, blood flow declined and "eventually reached baseline". During intermittent therapy, when the suction was turned on and off repeatedly, the increase in peak blood flow declined when the "off" interval was shorter than two minutes, whereas longer intervals had no effect on peak blood flow. Consequently, a 5-minute-on/2-minute-off cycle was considered optimal and was advocated for intermittent NPWT.

Many researchers have investigated the effects of NPWT on local blood flow, and both increases [36, 37, 56, 59] and decreases [36, 37, 52] have been reported. It is evident that although several studies have been performed on the changes in local blood flow due to NPWT, the results are not consistent, and it has therefore been recommended that further studies should be undertaken [33]. In the work described in this thesis, studies were, therefore, carried out to investigate the effects of NPWT on periwound blood flow in a peripheral porcine wound model, using both transcutaneous and invasive laser Doppler flowmetry, and thermodiffusion (Studies I, II, and III).

Wound contraction

One of the fundamental effects of NPWT is believed to be the macrodeformation of the wound edge tissue as the wound contracts [33]. This mechanical effect is thought to result in shearing forces at the wound–dressing interface, that affect the cytoskeleton [51], initiating a signaling cascade that ultimately leads to granulation tissue formation and wound healing.

The type of wound filler used for NPWT affects the degree of wound contraction. Foam results in greater wound contraction than gauze [60], probably because foam has a more open cell structure, while gauze is a woven, denser material. It has been shown previously that patients undergoing NPWT with foam are ~2.5 times more likely to show a reduction in wound area after one week of therapy than patients undergoing standard moist wound therapy [61]. Wound volume reduction, which may reach a weekly rate of 23% of the total wound volume [62], is achieved by a combination of increased granulation tissue formation and wound contraction. The mechanical effects of NPWT may be important for the entire wound-healing process,

as early changes in the size of a wound have been shown to correlate with final wound healing [61].

The effect of the wound filler (foam or gauze) on wound macrodeformation was examined in Study IV. In Study V, the impact of the negative pressure level on macrodeformation was studied.

Microdeformation

In terms of NPWT, microdeformation (also known as microstrain) refers to the transfer of an imprint of the surface topography of the compressed wound filler onto the tissue surface. These imprints can easily be observed as undulations on the surface of tissue that has been exposed to NPWT when viewed through a microscope [63]. When using foam, it is believed that the tissue beneath the foam is drawn up into the pores, while the walls of the foam pores leave an imprint on the tissue, thereby creating strain on the cells [51, 54, 64-66]. It has been shown that the pore size of foam affects the degree of wound bed deformation [67].

Finite element modeling of NPWT has demonstrated that most of the stretched elements show deformations of 5 to 20% [51]. This level of strain is similar to the *in vitro* levels of strain shown to promote cellular proliferation [68, 69]. Importantly, the deformation predicted by the model was similar in morphology to the surface undulations observed in histological cross-sections of the wounds. The authors hypothesized that this deformation stretched individual cells, thereby promoting proliferation in the wound microenvironment.

The wound interface may consist of either the wound filler (foam or gauze) or a low-adherent wound contact layer that is placed underneath the wound filler to cover the wound bed. The tissue surface is affected by the structure of the wound interface, as it will trigger the cells to divide, and to rebuild and strengthen the tissue. It has been shown that only areas of the wound bed that are covered with the wound filler (foam or gauze) show evidence of microdeformation, whereas a smooth wound bed surface is seen under a wound contact layer [70]. Scherer *et al.* investigated the interaction of the dressing itself with the underlying tissue, the effect of the hypobaric pressure on tissue, the fact that the wound is sealed by a semipermeable dressing, and a combination of these in a murine model [71]. It was demonstrated that foam (with or without negative pressure) was essential for angiogenesis, whereas both foam and negative pressure were needed for cell proliferation.

The mechanical forces exerted by NPWT (both macro- and microdeformation) deform the extracellular matrix in cells, and in doing so, capitalize on the tension stress effect. Cells require tension to divide and proliferate [68, 72], and cells that are not stretched have been known to undergo apoptosis [68, 69, 73]. The cytoskeleton maintains cell structure, and regulates cell response to extracellular forces. When cells are subjected to external forces, integrins mediate the force across cell membranes,

and an activated local intracellular transduction response leads to focal adhesion assembly, cytoskeletal strengthening, chemical signaling cascades, and gene transcription. Mechanical tissue deformation induces the expression of angiogenic growth factors and receptors such as vascular endothelial growth factor (VEGF), VEGF receptors and angiopoietin system receptors [54, 65, 74-79]. The stretching of endothelial cells *in vitro* leads to increased vessel formation [80, 81]. Deformation also induces the production of extracellular matrix components (proteoglycans, glycosaminoglycans, collagen and elastin) [65, 79, 82] and the down- and up-regulation of numerous biochemical markers and signaling factors [65, 83, 84]. In Study IV, the microdeformational properties of foam and gauze were compared.

Wound fluid removal
Moisture balance is essential in all phases of wound healing [85-87]. A moist wound environment accelerates the reepithelialization process, facilitates the action of growth factors, increases keratinocyte and fibroblast proliferation, enhances collagen synthesis, and promotes angiogenesis and early wound contraction [88-98]. While too little moisture can cause cell death, too much can promote maceration and damage to the wound edges and periwound skin. The challenge is to strike a balance to avoid extremes that can delay healing. Exudate removal is one of the mechanisms thought to promote wound healing during NPWT [17, 43, 44]. The exudate in a chronic wound may impair wound healing as the fluid contains elevated levels of inflammatory cytokines and proteolytic enzymes (as well as low amounts of growth-promoting cytokines) [99]. When wounds are slow to heal and become chronic, the composition of wound exudate is dominated by high levels of oxidative enzymes, cytokines, leukocytes, and proteases (e.g., matrix metalloproteinases [MMPs]), all of which impede healing [100-102]. NPWT affords continuous removal of wound fluid, which may reduce inhibitory factors [84]. In a recent study of eight adults receiving NPWT for pressure ulcers, it was shown that the levels of tumor necrosis factor-alpha in the wound fluid decreased significantly during the first 24 hours of treatment, and this decrease was sustained on day 7 [103]. Removing wound exudate by NPWT thus has a positive influence on the healing process. In Study V, the impact of the negative pressure level on fluid removal from the wound cavity was examined.

Therapy settings
NPWT can be tailored to a specific wound by means of the negative pressure level, the choice of wound filler, and the way in which the pressure is applied. When the present research started, little was known about the effect of NPWT settings on wound blood flow, wound contraction or fluid removal, and there were conflicting findings regarding pressure level [10, 104, 105], the effects on tissue compression [106-108], and changes in tissue blood flow [17, 35-37, 53, 56, 59, 104, 109-111].

Negative pressure level
A landmark study by Morykwas *et al.* [17], published in 1997, has, until now, been the basis for the way in which many clinicians apply NPWT. It was, for example, found in this study that –125 mmHg was the optimal pressure regarding local blood flow effects. Most NPWT devices are capable of delivering pressure levels ranging from –40 to –150 mmHg [112]. Thus, there is ample opportunity to use other pressure levels, although this is seldom done. Almost thirty years ago, Russian researchers published results indicating that the most effective level of negative pressure was –75 to –80 mmHg [12]. Today, many of the gauze-based NPWT device manufacturers recommend pressure settings of –60 to –80 mmHg. Little is known about the impact of different levels of negative pressure on the periwound blood flow and the degree of wound contraction. The effects of different levels of negative pressure on periwound blood flow, wound contraction and fluid evacuation were investigated in Study I and Study V, respectively.

Wound filler
The material used to fill the wound is called the wound filler. The properties of the filler in contact with the wound bed determine some of the effects of NPWT on the wound bed. The most common wound fillers used are polyurethane foam (average pore size 400 to 600 μm) [113] and gauze [112]. The porous structure of foam allows the exudate to be removed from the wound, while the more absorbent nature of gauze leads to more fluid being retained in the wound. Several groups maintain that the use of gauze is less painful to the patient than foam-based NPWT, and that the risk of bleeding and tissue damage is reduced [114-116]. Gauze is impregnated with polyhexamethylene biguanide, an antimicrobial agent. Until recently, polyurethane foam intended for use in NPWT did not have antimicrobial properties, but some foam has now been modified to contain micro-bonded metallic silver. The macro- and microdeformational effects of NPWT using foam and gauze on the wound were compared in Study IV.

Continuous, intermittent and variable negative pressure
In NPWT, the negative pressure is most commonly applied in the "continuous mode", i.e., the pressure is constant. However, the negative pressure can be repeatedly switched on and off, alternating between atmospheric and subatmospheric pressure; this is called "intermittent pressure" therapy. Treatment is usually applied in 5-minute-on/2-minute-off cycles, based on a study by Morykwas *et al.* [17]. It has been shown that the amount of granulation tissue formed in the wound bed is greater during intermittent NPWT [17, 117] compared to continuous therapy. This may be the result of both mechanical stimulation of the wound bed [117] and altered blood flow, which may enhance tissue oxygenation and angiogenesis.

Intermittent pressure therapy entails sudden changes in pressure that cause the foam to expand and contract over the granulation tissue, which may lead to pain. "Variable

pressure" therapy has, therefore, been introduced, providing smoother variations between the two different levels of negative pressure, thereby maintaining a negative pressure throughout the treatment [118]. It is not known if these two NPWT settings have the same effects on the wound. The impact of the NPWT application mode on periwound blood flow was investigated in Study III. Figure 3 (below) illustrates the various modes of pressure application.
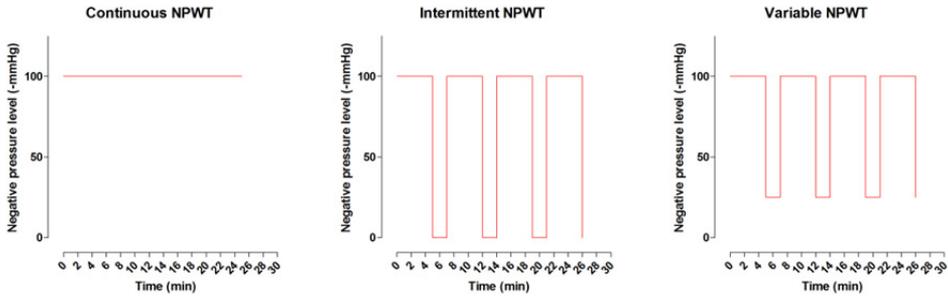
Figure 3. The three different modes of NPWT application: continuous, intermittent, and variable therapy.

Aims

The general aim of the work presented in this thesis was to study the biological effects of NPWT when using different levels of negative pressure, different wound filling materials (foam or gauze) and different ways of applying the negative pressure (continuously, intermittently or variably). The intention was to provide a scientific basis for the choice of NPWT parameters for the treatment of wounds.

The specific aims were:

- to examine the effects of NPWT on periwound blood flow using different levels of negative pressure and mode of pressure application (continuous, intermittent or variable NPWT),
- to examine the impact of different measurement techniques, including transcutaneous laser Doppler flowmetry, invasive laser Doppler flowmetry, and thermodiffusion, on periwound blood flow during NPWT,
- to examine the mechanical effects, including micro- and macrodeformation, of NPWT using different types of wound fillers (foam or gauze) and a wide range of negative pressure levels, and
- to examine fluid removal from the wound cavity when using different levels of negative pressure.

Methods

Study	Purpose	Methods	n
I	To measure the effect of the negative pressure level on blood flow at 0.5, 2.5, and 5 cm from the wound edge	Invasive LDF Continuous gauze-based NPWT	8 pigs*
II	To examine the impact of NPWT on blood flow at 0.5, 1, and 2.5 cm from the wound edge using different measurement techniques	Transcutaneous LDF, invasive LDF, and thermodiffusion Continuous foam-based NPWT	8 pigs
III	To examine the periwound blood flow changes during intermittent and variable NPWT	Invasive LDF Intermittent and variable gauze-based NPWT	8 pigs*
IV	To examine the mechanical effects, including micro- and macrodeformation, of NPWT using different types of wound fillers (foam or gauze)	Histological analysis + wound diameter measurements Continuous foam- and gauze-based NPWT	8 pigs
V	To determine the effect of the negative pressure level on wound contraction and fluid removal from the wound cavity	Wound diameter measurements + gravimetrical measurement of fluid removal Continuous gauze-based NPWT	16 pigs*

Overview of Studies I–V with regards to the purposes and the methods used. LDF = laser Doppler flowmetry, NPWT = negative pressure wound therapy. * The same eight pigs were used for Studies I, III and V.

Animal preparation and surgical procedure

A peripheral wound model in pigs was used in the experiments. Pigs were chosen for these studies as their cutaneous anatomy is similar to that of humans. Indeed, wound healing models in pigs have previously shown a striking 78% concordance with human studies [119]. The advantage of an acute wound model in animals is that the size, duration, location, and etiology can be standardized, and the conditions can be carefully controlled, in contrast to chronic wounds. However, animal subjects are likely to be younger and healthier than humans with wounds, they will not suffer from co-morbidity, and will not be exposed to other medical interventions during the course of the study.

Circular wounds (5 cm in diameter) extending through both subcutaneous and muscle tissue (2 cm deep) were created on the pigs' backs. The animals were anesthetized for the entire duration of the experiments. Detailed descriptions of the anesthesia can be found in the respective papers. The wounds were treated with NPWT. Saline-soaked AMD gauze (Studies I, III, IV and V) or polyurethane foam (Studies II and IV) were used as wound fillers. A drainage tube was inserted into the wound filler. The open wound was then sealed with a transparent adhesive drape. The drainage tubes were connected to a vacuum source, which could be set to deliver a freely adjustable negative pressure.

The studies were approved by the Ethics Committee for Animal Research at Lund University, Sweden.

Microvascular blood flow measurements

Studies I, II, and III were performed to investigate the effects of NPWT on blood flow in and around the wound edge.

Study I was performed to examine the effects of gauze-based NPWT on periwound blood flow when using levels of negative pressure ranging from –10 to –175 mmHg. Invasive laser Doppler flowmetry was used at lateral distances of 0.5, 2.5, and 5 cm from the wound edge since it is known from previous studies performed by our group that the major changes in blood flow during NPWT occur close to the wound edge (i.e., within a few centimeters) [36, 37]. Probes were placed both subcutaneously and in muscle tissue since we hypothesized that the tissue density would affect the blood flow (Figure 4).

Study II was designed to investigate the effects of foam-based NPWT on periwound blood flow using transcutaneous laser Doppler flowmetry, invasive laser Doppler flowmetry, and thermodiffusion. Probes were placed 0.5, 1 and 2.5 cm laterally from

the wound edge. Blood flow was recorded before and after NPWT was applied at different levels of negative pressure (–20, –40, –80 and –125 mmHg).

Study III aimed to examine the effects of intermittent and variable NPWT on periwound blood flow. Invasive laser Doppler flowmetry was used for blood flow measurements. The pressure levels used were –10 mmHg (minimal blood effects), –45 mmHg (half maximal blood flow effects), –75 mmHg (~maximal blood flow effects), and –125 mmHg (the previously recommended pressure level), and blood flow measurements were made 0.5 and 2.5 cm from the wound edge.

Invasive laser Doppler flowmetry

Laser Doppler flowmetry is a technique used to quantify the motion of red blood cells in a specific volume, and it has been applied extensively for the measurement of blood flow in flaps during plastic surgery procedures [120]. A fiberoptic probe carries a beam of laser light. Light reflected from moving blood cells undergoes a change in wavelength (Doppler shift), while light reflected from static objects is unchanged. The magnitude and frequency distribution of these changes in wavelength are directly related to the number and velocity of the blood cells. The information is collected by a returning fiber, converted into an electronic signal and analyzed. The principle is illustrated in Figure 4. In the current experiments, a system consisting of several parallel probes (PeriFlux System 5000, with 418-1 Probes, Perimed, Stockholm, Sweden; Figure 5) was used for measurements of the microvascular blood flow at and around the edge of the wound. The laser Doppler signal was monitored continuously on a computer screen (Figure 6).
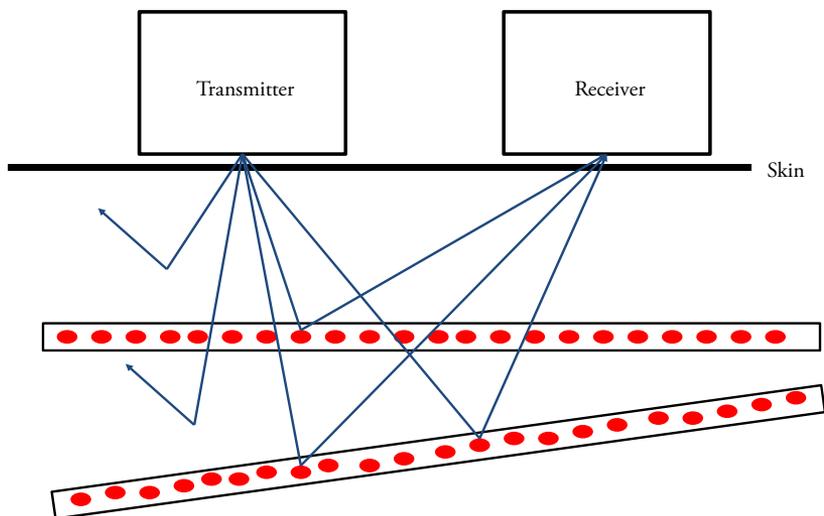
Figure 4. Illustration of the principle of laser Doppler flowmetry. When the tissue is illuminated by a laser source (transmitter), 93–97% of the light is either absorbed by various structures or undergoes scattering. The remaining 3–7% is reflected by moving red blood cells and returns to the second optical fiber (receiver). Microvascular blood flow is defined as the product of mean red blood cell (RBC) velocity and mean RBC concentration in the volume of tissue illuminated by the probe. (Adapted from [121].)
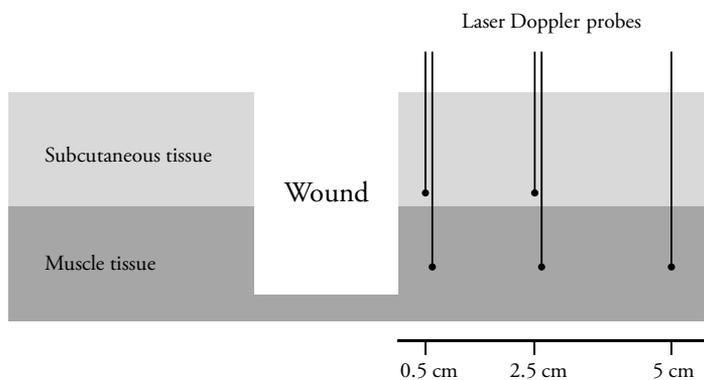
Figure 5. Illustration of the laser Doppler probe placement in Study I. Probes were placed in subcutaneous and muscle tissue at three different distances from the wound edge (0.5, 2.5, and 5 cm).
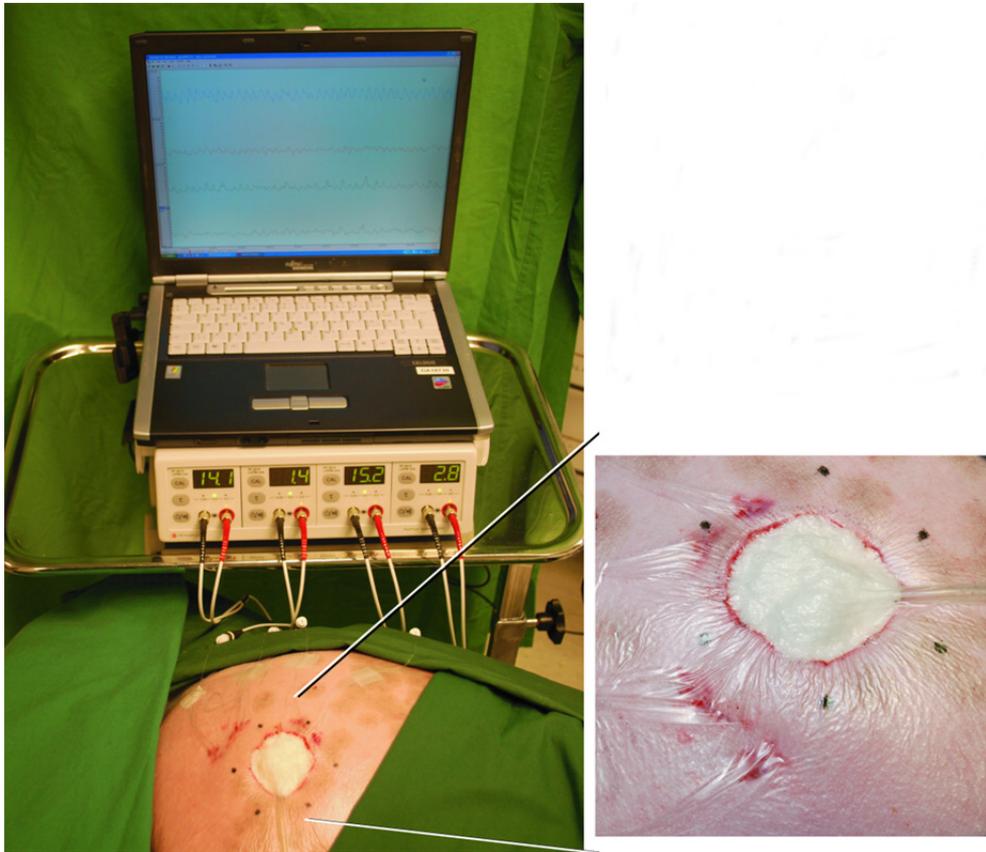
Figure 6. Periwound blood flow measurements using a multichannel PeriFlux System 5000 and fiberoptic probes. The signal is monitored in real time on the computer screen.

Transcutaneous laser Doppler flowmetry

Study II was performed using transcutaneous laser Doppler flowmetry. The equipment used (O2C unit, LEA Medizintechnik, Giessen, Germany) transmits both continuous laser light and white light into the tissue, where it is scattered and collected at the skin surface. The collected light is split into its spectral components by a charge-coupled device, and is then converted into an electrical signal. The laser Doppler shift is detected, and the product of moving RBCs and the velocity of each RBC is used to calculate the relative blood flow. White light is used for the detection of oxygen saturation and the relative amount of hemoglobin. Blood flow and the relative amount of hemoglobin are obtained in terms of arbitrary units, whereas oxygen saturation is expressed in percent.

33

Flat measurement probes (LF-2, LEA Medizintechnik, Giessen, Germany) were placed on the skin surface and held in place by a transparent adhesive film (Figure 7). In the present studies, only the laser Doppler measurements were used.
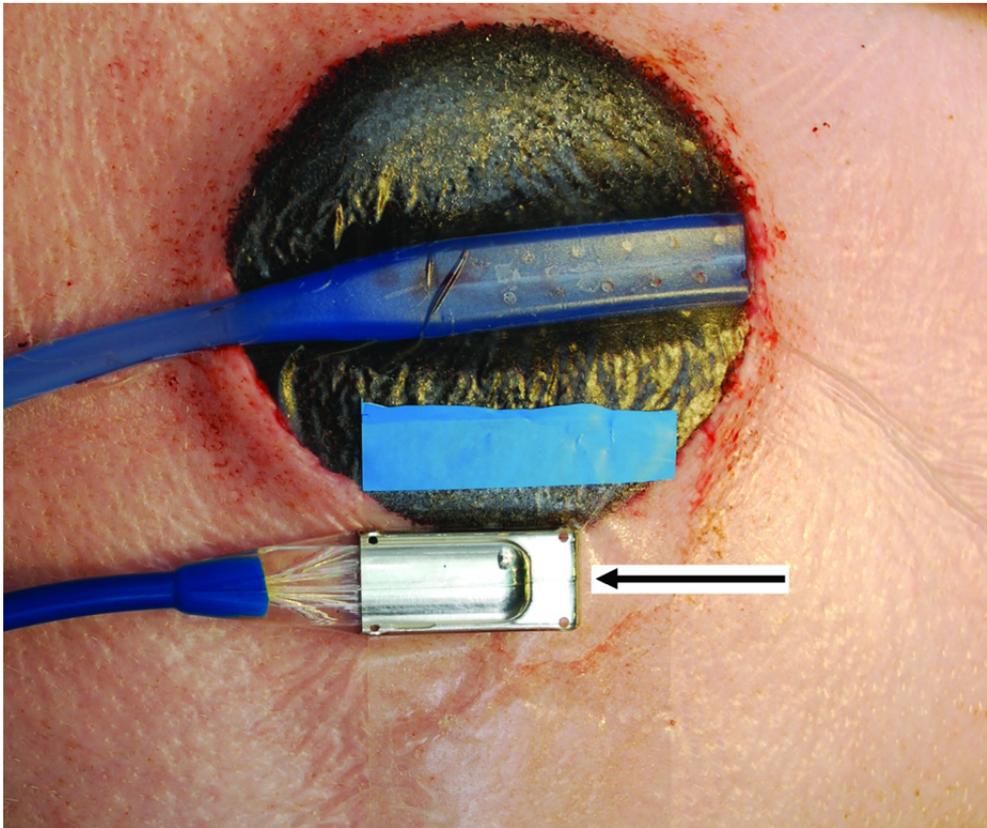
Figure 7. Foam-based NPWT with the transcutaneous laser Doppler probe placed on the skin (arrow).

Thermodiffusion

In Study II, blood flow was measured at the wound edge using the Bowman Perfusion Monitor (Hemedex, Cambridge, MA, USA), which is based on the principle of thermodiffusion. A thermal transducer (Figure 8) is mounted at the tip of the flexible 19G QFlow 500 Thermal Diffusion Probe. The transducer is heated to a temperature 2 °C above that of the surrounding tissue. The power dissipated in the thermistor provides a measure of the ability of the tissue to remove the heat by both thermal conduction within the tissue and by thermal convection due to tissue blood flow. A passive, proximal thermistor is used to monitor, and compensate for,

temporal changes in baseline tissue temperature. Perfusion is expressed as ml/100 mg tissue/min in a small focal volume of tissue surrounding the distal tip of the probe.
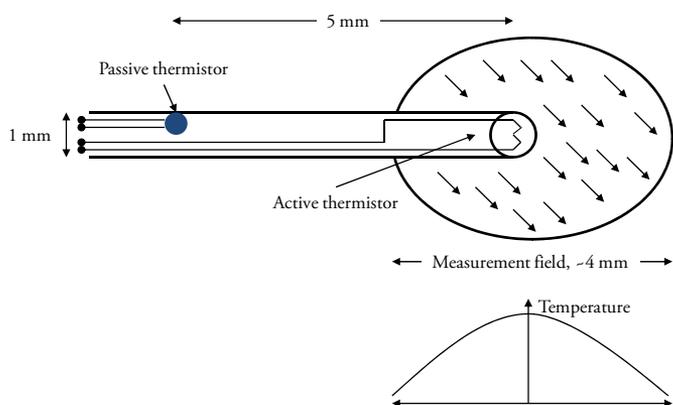
Figure 8. Schematic of the thermal diffusion probe showing the active, heated thermistor at the probe tip, which produces a thermal measurement field in the surrounding tissue. The size of the thermal measurement field is dependent on the thermal properties of the tissue and the blood flow; high blood flow produces a smaller thermal field. The diameter of the field is approximately 4 mm for typical values of thermal properties and blood flow. The passive thermistor, mounted 5 mm proximal to the probe tip, monitors the variations in baseline tissue temperature.

Histology

In Study IV, a histological examination of the wound bed tissue was performed. A piece of the wound filling material (foam or gauze) was sutured to the bottom of each wound. NPWT at –75 or –125 mmHg was applied continuously for 72 hours. After discontinuation of NPWT, the strip of wound filler and the underlying wound bed tissue were cut loose and prepared for histological examination. The specimens were separately stained with both giemsa and hematoxylin-eosin, and analyzed with regard to wound bed surface undulations and the microdeformational effects of NPWT.

Wound contraction

In Studies IV and V, wound contraction resulting from NPWT was investigated. Measurements of the size of the wound were made using a slide caliper. In Study IV, four marks were made around the edge of the wound and in Study V, six marks were made around the edge of the wound (Figure 9). The diameters of the wound were

measured before and after the application of negative pressure (–75 or –125 mmHg in Study IV, and –10 to –175 mmHg in Study V), and the mean change in diameter was calculated.
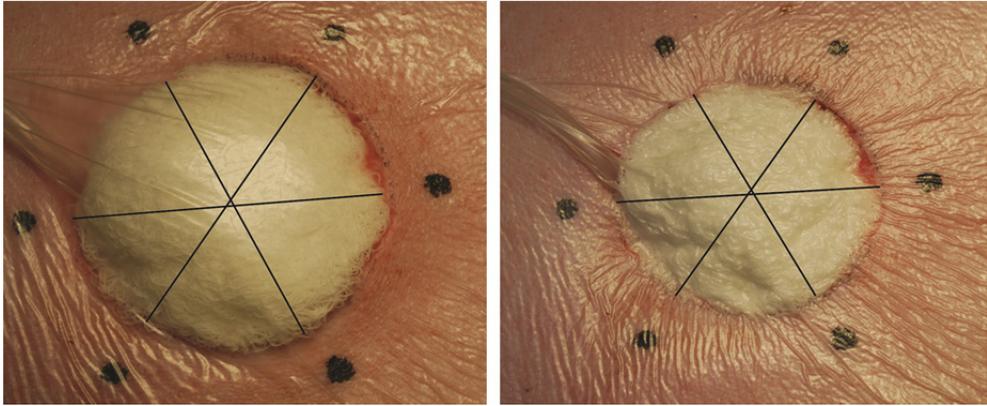
Figure 9. Illustration of how the measurement of the wound diameter was performed in Study V. Every diameter was recorded before (left) and after (right) application of NPWT, and the average change was then used for the statistical analysis.

Statistical analysis

In Studies I–IV, statistical analysis was performed using the Mann–Whitney test when comparing two groups, and the Kruskal–Wallis test with Dunn's test for multiple comparisons when comparing three groups or more. In Study V, statistical analysis was performed using the Wilcoxon matched-pairs signed rank test. Significance was defined as $p<0.05$. Non-parametric tests were used for statistical testing as the studies were based on a small number of observations. Mean values and the standard error of the mean were used to describe the data in the articles. However, it would have been better to present this non-normally distributed data as median values and percentiles, or in scatter plots. Thus, the data is presented in scatter plots with median values in the thesis. This does not affect the conclusions of the thesis.

Results and Discussion

The effects of NPWT on blood flow

Level of negative pressure

The results of Study I showed that the microvascular blood flow at the wound edge changes gradually with increasing level of negative pressure, and then reaches a plateau at higher levels of negative pressure. The greatest increase in blood flow was seen at about –80 mmHg, while half this effect was seen at approximately –45 mmHg. Levels of negative pressure greater than –80 mmHg (e.g., –125 mmHg) did not further increase the blood flow. Closer to the wound edge, the decrease in blood flow was most pronounced near –90 mmHg, whereas half this effect was achieved at about –30 mmHg.

The most commonly used pressure level in treating humans, –125 mmHg, is based on a limited study on pigs carried out in 1997 [17]. However, the current results showed that maximal blood flow effects were obtained at about –80 mmHg. The level of negative pressure can, thus, be tailored to balance the local blood flow effects. When using NPWT for the treatment of poorly perfused tissue (e.g., diabetic foot ulcers and thin skin transplants), ischemia can develop at the wound edges. There is also a risk that the patient will find the treatment painful [57]. In such cases, it may be advantageous to reduce the negative pressure to, e.g., –40 mmHg, which is the level at which about half the maximal blood flow effect was seen. Indeed, clinical studies have confirmed that negative pressure levels of less than –125 mmHg may result in excellent wound healing [57]. Further clinical studies are needed to corroborate the findings before recommendations can be made.

Increase in blood flow 2.5 cm from the wound edge

In Studies I, II, and III, an increase in blood flow was seen at a distance of 2.5 cm from the wound edge upon the application of NPWT (Figure 10). The mechanism causing this is not clear. Kairinos *et al.* showed that the pressure in the tissue at distances greater than 2 cm from the wound edge was the same as atmospheric pressure [122]; thus, altered pressure on the tissue does not seem to explain the present findings. One plausible explanation could be the mechanical effects that cause

wound contraction, as shown in a previous study by our group [50] and in Studies IV and V. When applied to a wound cavity, as in the present study, NPWT will create a pulling force on the tissue that may open up vascular beds, increasing blood flow. Indeed, previous experimental studies have shown that small arterioles and capillaries in the tissue around the wound edge open upon the application of negative pressure [53, 123]. It is believed that the NPWT-associated increase in periwound blood flow is important for oxygenation, nutrient supply and the removal of waste products from the healing wound [124]. Also, increased blood flow may facilitate the penetration of antibiotics to otherwise poorly perfused tissue.

Figure 10. Blood flow changes in the periwound tissue (both subcutaneous and muscle tissue) 2.5 cm from the wound edge upon application of NPWT (Study I, *n* = 8). Measurements were made with invasive laser Doppler flowmetry. The red line denotes the median value.

Decrease in blood flow 0.5 cm from the wound edge

Measurements using invasive laser Doppler flowmetry showed that the application of NPWT causes a decrease in blood flow 0.5 cm from the wound edge (Studies I, II and III) (Figure 11). The mechanism by which NPWT decreases blood flow in superficial tissue may have been identified in recent work [122, 125]. When the wound contracts, the tissue at the wound edge collapses towards the suction force, and the pressure around the rim of the wound edge increases [122], leading to a decrease in blood flow. It was shown by Kairinos *et al*. [122] that increasing the

suction pressure in NPWT leads to gradually increasing tissue pressure, which explains the present finding that blood flow changes gradually with increasing negative pressure.

There are both advantages and disadvantages of the hypoperfusion caused by NPWT. It is well known that reduced blood flow stimulates angiogenesis and granulation tissue formation, which in turn facilitate the process of wound healing [43, 110]. However, several clinical problems are associated with the hypoperfusion caused by NPWT. In tissues with already impaired blood flow, e.g., thin tissue grafts and diabetic foot ulcers, the decrease in blood flow may result in ischemia, and it has been suggested that NPWT should be applied with great caution to tissues with compromised vascularity [52]. Some advocate that NPWT is contraindicated if there is any doubt about the vascularity of the tissue [126, 127]. The choice of negative pressure level must, therefore, be based on the type of wound and its vascularity.
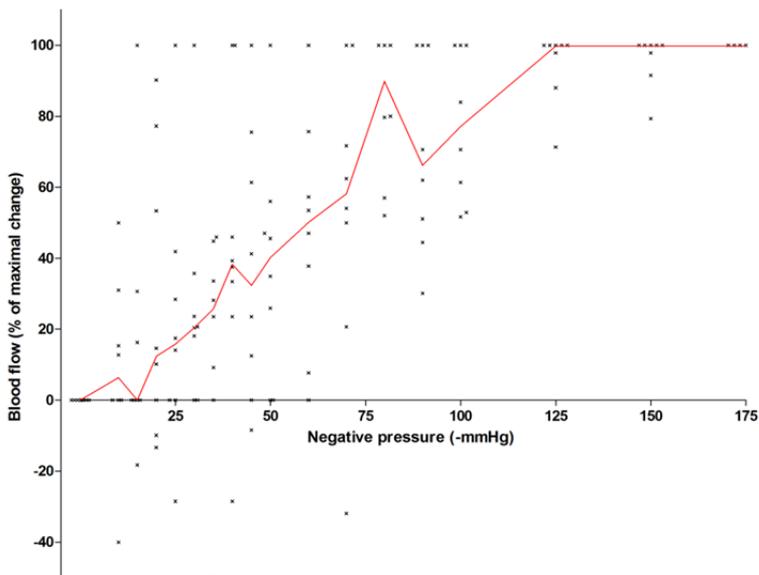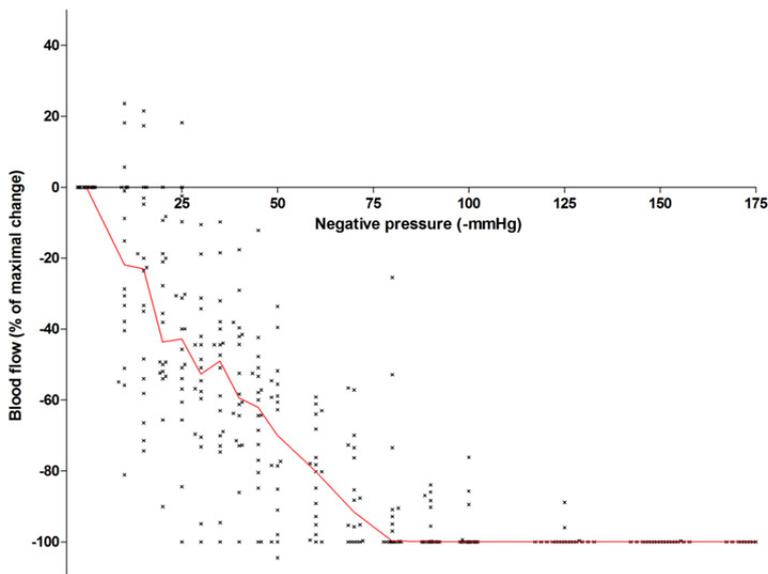

Figure 11. Blood flow changes in the periwound tissue (both subcutaneous and muscle tissue) 0.5 cm from the wound edge immediately after application of NPWT (Study I, n = 18). Measurements were made with invasive laser Doppler flowmetry. The red line denotes the median value.

Effects on blood flow 1 cm from the wound edge – the transition zone

The effect on blood flow 1 cm from the wound edge was investigated in Study II. Low pressure levels led to increased blood flow, while high levels of negative pressure resulted in decreased blood flow. This indicates that there may be a transition zone

between the hypoperfused tissue closer to the wound and the hyperperfused tissue further from the wound. Thus, the blood flow response when NPWT is applied depends on the level of negative pressure, as shown in Figures 12 and 13 below.
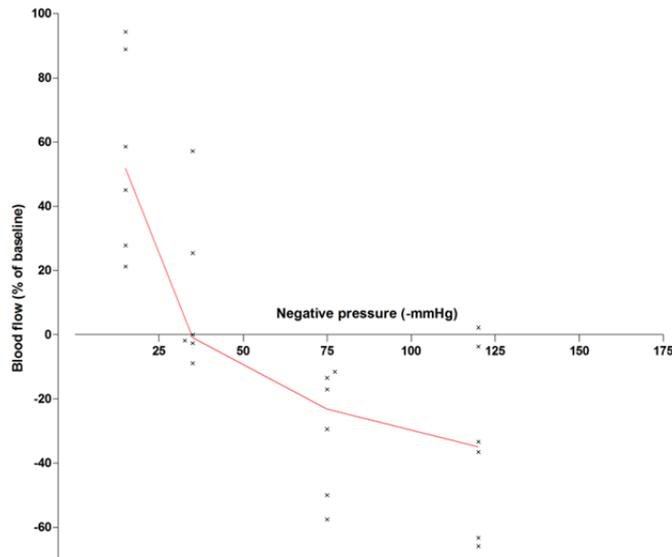
Figure 12. Blood flow changes in muscle tissue 1 cm from the wound edge immediately after application of NPWT (Study II, n = 6). Measurements were made with invasive laser Doppler flowmetry. The red line denotes the median value.
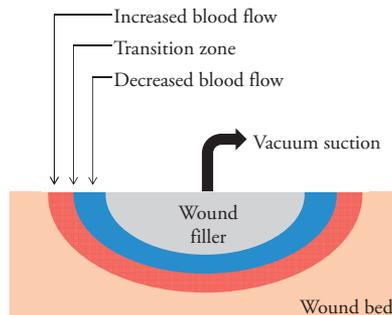
Figure 13. Illustration of the observed periwound blood flow changes in a cross-section of a peripheral wound. Close to the wound there is a decrease in blood flow, whereas blood flow increases further away. At a distance of 1 cm from the wound there seems to be a transition zone between hypo- and hyperperfused tissue.

Comparison of wound edge blood flow changes using transcutaneous laser Doppler flowmetry, invasive laser Doppler flowmetry, and thermodiffusion

Several studies have been performed on NPWT-induced changes in local blood flow. The results are not consistent, and both increases [36, 37, 56, 59] and decreases [36, 37, 52] have been reported and it has therefore been recommended that further studies should be undertaken [33]. In the work described in this thesis, studies were, therefore, carried out to investigate the effects of NPWT on periwound blood flow in a peripheral porcine wound model, using a number of techniques in parallel.

The results of blood flow measurements 0.5, 1 and 2.5 cm from the wound edge, using three different techniques (transcutaneous and invasive laser Doppler flowmetry, and thermodiffusion) at negative pressures of –20, –40, –80, and –125 mmHg (Study II) indicated that the results were partly dependent on the measurement technique used. Thermodiffusion (an invasive measurement technique) generally showed a decrease in blood flow close to the wound edge (0.5 cm), and an increase 2.5 cm from the wound edge. Invasive laser Doppler flowmetry showed a similar response pattern, with a decrease in blood flow 0.5 cm from the wound edge and an increase further away. A different response pattern was seen with transcutaneous laser Doppler flowmetry, showing an increase in blood flow regardless of the distance from the wound edge.

The reason for the disparity between invasive and transcutaneous laser Doppler measurements may be that these techniques measure blood flow in different types of tissue (i.e., the dermis in the case of the transcutaneous technique, and muscle tissue in the case of the invasive technique). The effect of NPWT on the blood flow in tissue close to the wound edge may therefore be the result of tissue compression, resulting in an increase in tissue pressure [122]. Dense tissue such as pig skin may resist compression, and no decrease in blood flow may be seen there, while muscle tissue is less dense and will be compressed to a greater extent, resulting in decreased blood flow during NPWT. Another explanation of the differences between the subcutaneous and transcutaneous measurements may be that, for the transcutaneous technique, the laser light must travel through the superficial skin layers before reaching the microcirculation, while probes inserted into the tissue will be in direct contact with the microvasculature of the tissue. The invasive techniques used entail the insertion of a space-occupying object into the tissue, thereby generating an increase in tissue pressure around the probe. It is of outermost importance that blood flow measurements using invasive techniques are performed using a probe that is as thin as possible. To summarize, it is impossible to judge which of these techniques that most accurately reflects the real situation. Transcutaneous measurement has been the method of choice in clinical practice due to the non-invasiveness of the technique, but the results should be interpreted with caution.

Limitations of the blood flow measurement techniques

Laser Doppler flowmetry is based on the fact that light impinging on moving objects undergoes a change in wavelength (Doppler shift), while light hitting static objects is unchanged. Thus, if the tissue under investigation moves, a much higher signal will be obtained than that caused by blood flow itself. This means that artifacts will be introduced by movement resulting from, e.g., breathing and NPWT-induced wound contraction, and care must be taken to eliminate all sources of motion. As the laser Doppler signal was continuously monitored on a computer screen, obviously erroneous signals could be seen immediately.

A limitation of the thermodiffusion method stems from the assumption that the tissue is a continuum of blood vessels with a uniform distribution of blood flow in the tissue within a few millimeters of the probe tip. This assumption is valid when all the vessels within the field of measurement of the sensor have a smaller diameter than the diameter of the sensor. However, an individual vessel can affect the measurements if it is large enough and close enough to the sensor. These vessels are called thermally significant. Fortunately, when such a vessel is present, its existence is evident from the data and the probe can either be repositioned or the data discarded.

Effects of intermittent and variable NPWT on blood flow

The aim of Study III was to examine the effects of intermittent and variable negative pressure on blood flow. The pressure settings used in Study III were based on the results obtained in Study I, where maximal effect on blood flow was seen at –80 mmHg. A low negative pressure, e.g., –10 mmHg, had only minimal effects on blood flow, whereas –45 mmHg resulted in approximately half the maximal effect on blood flow. The clinical golden standard has been –125 mmHg, but –75 mmHg has almost the same effects on blood flow (Study I). In Study III, intermittent therapy was administered by alternating the pressure from 0 to –75 and from 0 to –125 mmHg, while variable therapy was administered by alternating the pressure between –10 and –75 and between –10 and –125 mmHg, and between –45 and –75 and –45 and –125 mmHg. Each cycle comprised five minutes of high pressure and two minutes of low pressure. Blood flow in subcutaneous and muscle tissue was measured 0.5 and 2.5 cm from the wound edge using laser Doppler flowmetry.

The results of Study III show repeated changes in blood flow as the negative pressure is alternated. A greater difference between the pressures (e.g., from –10 to –75 mmHg) results in greater changes in blood flow than smaller pressure changes (e.g., from –45 to –75 mmHg). The combination of increased and decreased blood flow may be beneficial since an increase is known to facilitate oxygenation and nutrient supply, while decreased blood flow stimulates angiogenesis and granulation tissue formation. Alternating the negative pressure may be especially beneficial when

treating poorly vascularized tissue. In cases where intermittent therapy causes patient discomfort due to pain, variable therapy may be a viable option.

Interestingly, no differences were seen in blood flow patterns between intermittent and variable NPWT with the high pressure settings at –75 and –125 mmHg. This may be explained by a peak blood flow effect already at –75 mmHg, and thus further elevation of the negative pressure would not cause additional effects on blood flow, as was found in Study I. Furthermore, there is no apparent difference in the effect on blood flow when variable NPWT with a lower setting of –10 mmHg is compared to intermittent NPWT (i.e., a lower setting of 0 mmHg). This might be explained by the fact that –10 mmHg has only a minimal effect on blood flow (see Study I).

Alternating effects on periwound blood flow can thus be obtained by varying the level of negative pressure during NPWT, as in variable and intermittent therapy. Close to the wound edge, the blood flow decreases when NPWT is switched to the higher pressure setting (e.g., –75 or –125 mmHg) and returns to baseline when NPWT is switched to the lower pressure setting, e.g., 0 or –10 mmHg. Further from the wound, the effects will be the opposite; blood flow increases when a high negative pressure is applied and returns to baseline when switched to the lower pressure setting (0 or –10 mmHg). Indeed, Morykwas and coworkers showed that intermittent NPWT resulted in a 103% increase in new granulation tissue formation compared to a more modest 63% increase during continuous NPWT [17]. Venturi *et al.* suggested that intermittent negative pressure was more effective than continuous negative pressure in stimulating wound healing because intermittent pressure causes greater cell deformation [126].

Mechanical effects of NPWT

One of the fundamental effects of NPWT is widely believed to be the deformation of the wound edge tissue. These mechanical effects affect the cytoskeleton [51], initiating a signaling cascade that ultimately leads to granulation tissue formation and wound healing. The level of negative pressure necessary to optimize the mechanical effects of NPWT is not known, and the use of a standard suction pressure of –125 mmHg on all wounds has been questioned [128]. Neither are the effects of different wound filler materials known. Too high a degree of contraction may have clinical disadvantages, e.g., patients may experience pain during the treatment [56, 129] and there is also the risk of ischemia in wounds with compromised vascularity [52].

Macrodeformation

In Studies IV and V, the effects of NPWT on macrodeformation were studied with regard to the type of wound filler (foam or gauze) and negative pressure level. The reduction in wound surface diameter was measured using a slide caliper. The long-term effects of NPWT were investigated in Study IV. NPWT was applied continuously for 72 hours at 0, –75 and –125 mmHg, using either foam or gauze as wound filler. In Study V, the immediate effects of different levels of negative pressure (–10 to –175 mmHg) on wound contraction were investigated.

Type of wound filler

In study IV, it was found that both foam and gauze resulted in a similar degree of wound contraction for the entire duration of the therapy (72 h; Figure 14). This augments the results from other studies, where the immediate effects of NPWT on wound contraction were similar regardless of whether foam or gauze was used [50, 130]. Others have reported that wound contraction is greater with foam-based NPWT compared to when using gauze [53, 131, 132]. It has been shown that in large wounds, where the difference in wound contraction between different wound fillers is more apparent, foam results in greater wound contraction than gauze [133]. This is probably due to foam having an open cell structure, being less dense than gauze, thereby allowing greater compression and volume reduction.
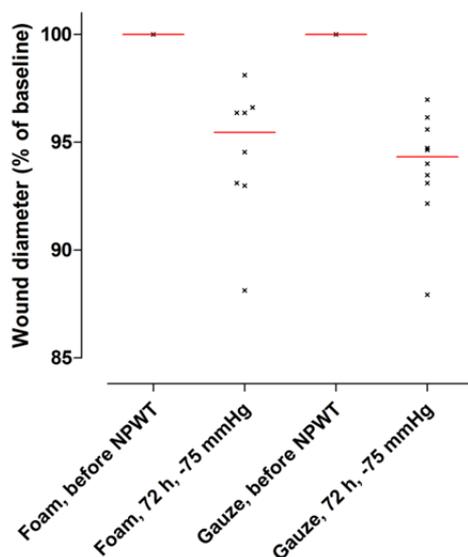
Figure 14. The effect of the choice of wound filler on wound contraction after 72 hours of continuous NPWT (*n* = 8). The red line denotes the median value.

Level of negative pressure

Results from Study V show that the degree of wound contraction increases gradually with increasing negative pressure and then stabilizes around –75 mmHg (Figure 15). Furthermore, the mechanical effects of NPWT at –75 mmHg are similar to those at –125 mmHg, which is in accordance with Study IV where the degree of macrodeformation was the same at –75 and –125 mmHg. A negative pressure of approximately –25 mmHg causes half maximum wound contraction. The finding that NPWT causes a reduction in wound surface diameter is supported by previous work [53, 132, 134, 135]. Isago *et al.* demonstrated that maximum wound area reduction was seen already at –50 mmHg [135], thus corroborating the present findings. It has been demonstrated that a reduction in the surface area of the wound after one week of NPWT predicts complete wound healing, and patients treated with NPWT are ~2.5 times more likely to show a significant wound area reduction one week after initiation of therapy than patients receiving standard moist wound therapy [61].
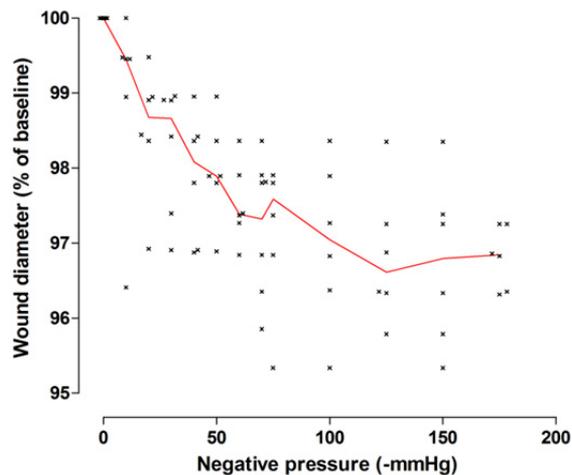
Figure 15. The immediate effect of the negative pressure level on the change in wound diameter, i.e., wound contraction (*n* = 6). The red line denotes the median value.

Microdeformation

In Study IV, the effects of the type of wound filler (foam or gauze) and the negative pressure level on NPWT-induced microdeformation were examined by histological analysis of the wound bed tissue. Microdeformation of the wound bed has previously been reported when using foam-based NPW [51]. The results of Study IV show, for the first time, that gauze-based NPWT also results in microdeformation of the wound bed. Furthermore, the microdeformation induced by NPWT is similar for foam and gauze. In a study by Wilkes *et al.*, using a computerized model, it was predicted that a polyurethane foam dressing would produce higher levels of tissue microdeformation than gauze at commonly used levels of negative pressure [64]. One reason for the discrepancy in results may stem from the difference between the *in vivo* situation and computer modeling.

The histological analysis of the wound bed clearly showed that, for foam, the edges of the foam pores compressed the tissue, and small tissue blebs had been drawn into the pores (Figure 16, left). A similar pattern of surface undulations was seen with gauze (Figure 16, right). In this case, tissue blebs had been drawn into the spaces between the threads of the gauze.
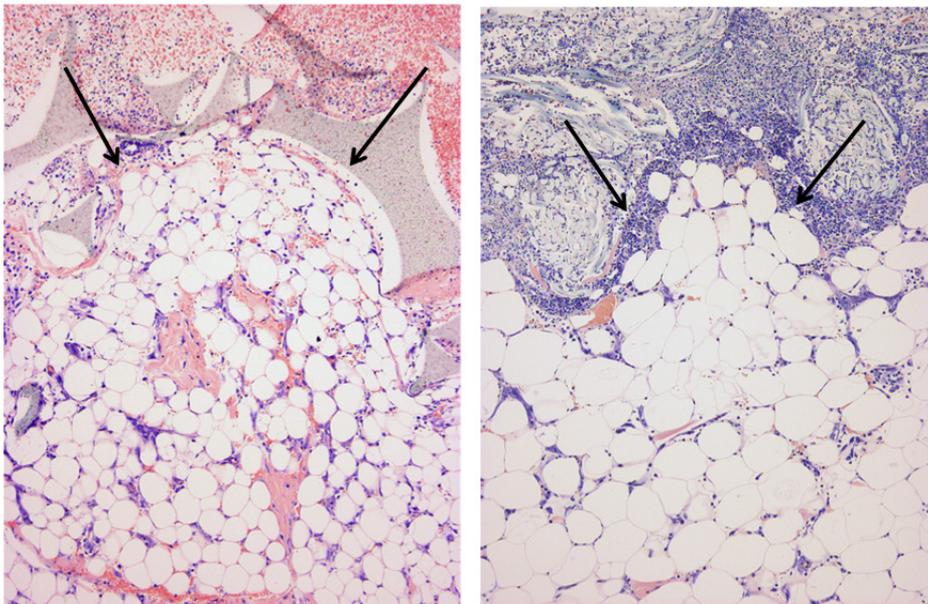
Figure 16. Giemsa-stained sections of the wound bed after 72 hours of continuous NPWT at –75 mmHg. Microdeformation of the wound bed is apparent both when using foam (left) and gauze (right). The arrows show the tissue imprints induced by the wound filler.

Saxena *et al.* suggested that the application of micromechanical forces to the wound edge may be the most significant mechanism of action of NPWT [51]. Significant microdeformation of the wound bed has been reported in wounds treated with NPWT using foam, showing protrusions and indentations corresponding to the structure of the foam, while tissue covered only with an occlusive dressing, and no foam, showed no undulations [51]. It has been calculated that most tissue elements are stretched by five to twenty percent by NPWT, which is similar to *in vitro* strain levels shown to promote cellular proliferation [51].

Micromechanical forces and cell deformation have been shown to cause a wide variety of molecular responses, including changes in ion concentration and permeability of membrane ion channels, release of secondary messengers, stimulation of molecular pathways, and alterations in gene expression resulting in cell proliferation and division [136]. It has been shown that cells made to stretch can divide and proliferate in response to soluble growth factors, whereas cells that are not stretched are cell-cycle arrested and tend to undergo apoptosis [68, 73].

Fluid evacuation by NPWT

Exudate removal is another mechanism thought to promote wound healing during NPWT [17, 43, 44]. Study V was, therefore, performed to investigate fluid removal at different levels of negative pressure. The amount of fluid removed was found to increase gradually with increasing level of negative pressure, until a steady state was reached (Figure 17). Maximal fluid removal was seen at approximately –125 mmHg, and half this at about –25 mmHg. Thus, in wounds with large volumes of exudate, a high negative pressure (–125 mmHg) may be needed initially. The reason for the discrepancy in the level of negative pressure required for maximum fluid removal and the other biological effects (blood flow, macro- and microdeformation) in the wound edge tissue cannot be deduced from the present study. However, there may be a difference between the effects of NPWT on the tissue compared to the effects on the wound space.
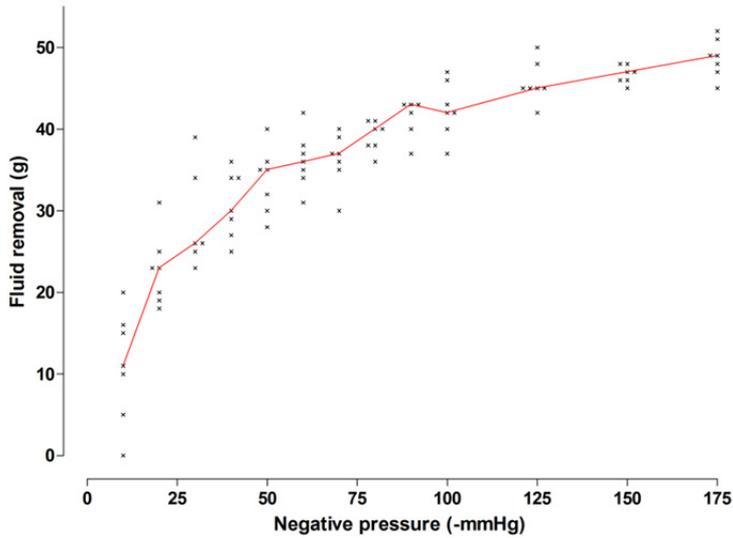
Figure 17. Fluid removal measured gravimetrically after 3 minutes of continuous NPWT ($n = 7$). The red line denotes the median values.

It is generally believed that higher levels of negative pressure are needed to treat wounds containing large volumes of exudate. The effect of NPWT on fluid removal has been shown to depend on the size of the cavity. Lindstedt *et al*. showed that treatment was required for approximately 6 minutes at –70 mmHg to reach maximal fluid removal in large abdominal wounds into which 500 ml albumin had been injected [137]. However, most NPWT-treated wounds are small, and Study V shows that most of the fluid is evacuated within 3 minutes of application of NPWT from a wound volume of ~60 cm^3 (Figure 18). There thus appear to be few reasons, if any, to increase the pressure level beyond –75 mmHg. In large volume wounds with large volumes of exudate, the need for a higher pressure setting will probably be temporary for the first day or two, after which the pressure can be reduced to levels more suitable for healing of the wound edge tissue (as discussed above). Further clinical studies are needed to corroborate the findings before recommendations can be made.
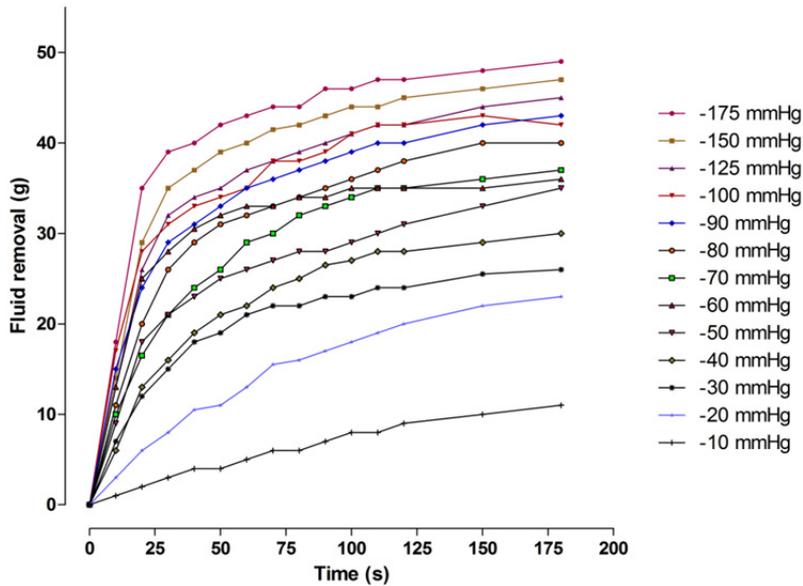
Figure 18. Median fluid removal for different pressure levels (*n* = 7).

Previous studies emphasize the importance of wound fluid removal as one of the beneficial effects of NPWT [17, 43, 44]. In a study on rabbits, optimum wound drainage occurred at a negative pressure level of –75 to –80 mmHg [138]. Chronic wound fluid is known to inhibit cell proliferation [139, 140], and chronic inflammation is associated with a vicious cycle in which inflammatory cells secrete cytokines, which in turn attract more inflammatory cells [141]. At the molecular level, this has been shown to be associated with an imbalance between the concentration of proteases (e.g., MMP-3, MMP-9), protease inhibitors (e.g., TIMP-1), and pro-inflammatory cytokines (e.g., tumor necrosis factor-alpha, interleukin-1 beta) [142-144]. The level of pro-MMP-9 (and active MMP-9) has been shown to be an accurate prognostic indicator of subsequent healing when measured in venous ulcers [145]. In a study by Scherer *et al.* it was found that pro-MMP levels were reduced during NPWT and during a 10-day follow-up period [21]. In a recent study of eight adults receiving NPWT for pressure ulcers, the levels of tumor necrosis factor-alpha in the wound fluid were found to decrease significantly during the first 24 hours, and this decrease was sustained on day 7 [103]. Removing wound exudate by NPWT, and thereby optimizing the microenvironment of the wound, most certainly has a positive influence on the healing process.

Conclusions

Effects of NPWT on blood flow

In Studies I and II it was found that blood flow decreased 0.5 cm from the wound edge and increased 2.5 cm from the wound edge. At 1 cm from the wound edge, low pressure levels led to increased blood flow, while high levels of negative pressure resulted in decreased blood flow. This may thus represent a transition zone between the hypoperfused tissue closer to the wound and the hyperperfused tissue further from the wound. The combination of hypo- and hyperperfusion may be beneficial in the process of wound healing as hyperperfusion facilitates oxygenation, nutrient supply, and removal of waste products, whereas hypoperfusion stimulates angiogenesis and granulation tissue formation.

Blood flow changed gradually with increasing level of negative pressure, reaching half maximum effect at approximately –45 mmHg and achieving a steady state at about –80 mmHg. Higher levels of negative pressure (e.g., –125 mmHg) had no further effect on blood flow. The optimal level of negative pressure for treating a wound may be the point at which steady state is reached, thereby lowering the risk of causing ischemia and pain. A lower level of negative pressure may be considered when treating poorly vascularized tissue or in patients suffering treatment-related pain (Study I).

The effects of NPWT on blood flow reported by different research groups show disparity. It has been speculated that the results depend on the measurement technique used. Study II was therefore designed to investigate the effects of NPWT on periwound blood flow using transcutaneous laser Doppler flowmetry, invasive laser Doppler flowmetry, and thermodiffusion. The results showed that the pattern of response may depend on the measurement technique. All three techniques indicated an increase in blood flow 2.5 cm from the wound edge. When using thermodiffusion and invasive laser Doppler, the blood flow decreased 0.5 cm from the wound, whereas an increase was seen with transcutaneous laser Doppler. The transcutaneous laser Doppler technique is the method of choice in clinical practice due to its non-invasiveness, however, the results should be interpreted with caution.

The beneficial effects of NPWT on wound healing have been suggested to be even greater if the level of negative pressure is varied during treatment, for example, intermittent or variable NPWT. Only a few studies have addressed the biological effects of intermittent NPWT. The results of Study III show repeated changes in

blood flow as the negative pressure is alternated. The combination of increased and decreased blood flow may be beneficial since an increase is known to facilitate oxygenation and nutrient supply, while decreased blood flow stimulates angiogenesis and granulation tissue formation. Alternating the negative pressure may be especially beneficial when treating poorly vascularized tissue. In cases where intermittent NPWT causes discomfort, variable NPWT may be a good alternative as it provides the same effects on periwound blood flow.

Mechanical effects of NPWT

In Studies IV and V, the effects of NPWT on macrodeformation (i.e., wound contraction) were studied with regard to the type of wound filler (foam or gauze) and the level of negative pressure. The results of Study V show that the degree of wound contraction changes gradually with increasing negative pressure level, reaching 50% of maximal wound contraction at around –45 mmHg and then reaching a steady state at approximately –75 mmHg. The mechanical effects at –75 mmHg were similar to those at –125 mmHg. These findings imply that –75 mmHg is a sufficient level of negative pressure to achieve maximal effects on wound contraction. As the negative pressure level often has to be reduced in the clinical setting, e.g., due to pain, or if the wound is poorly vascularized, it appears this can be done in the knowledge that this will not reduce the mechanical effects on the wound edge tissue. Even when the negative pressure was reduced to approximately –30 mmHg, there are still pronounced mechanical effects.

In Study IV, the effects of NPWT on microdeformation were investigated by histological analysis of the wound bed tissue. Microdeformation during NPWT has previously been shown for foam. The results of Study IV show that gauze-based NPWT also results in microdeformation of the wound bed. Micromechanical forces are known to induce cell proliferation and division, and this may be one of the key mechanisms of NPWT in accelerating wound healing.

Fluid evacuation by NPWT

Study V was performed to investigate fluid removal at different levels of negative pressure. The amount of evacuated fluid increased gradually with increasing levels of negative pressure until a steady state was reached. A negative pressure of –125 mmHg led to near-maximal fluid removal, and approximately –45 mmHg half maximum fluid removal. It may thus be beneficial to treat wounds containing large volumes of exudates at a high pressure (i.e., –125 mmHg) initially and then decrease the pressure to a level better suited to the needs of the wound edge tissue.

Clinical implications

Today, it is possible to tailor NPWT through the choice of negative pressure level, the wound filler material (foam or gauze), and the way in which the pressure is applied (continuously, intermittently or variably). The *in vivo* research described in this thesis investigates the effects of these variables on some of the biological processes involved in wound healing. The studies were carried out on pigs, but interestingly enough, experienced clinicians have empirically come to the same conclusions. However, further clinical studies are needed to corroborate the findings before clinical recommendations can be made.

Negative pressure level

The most commonly used pressure level, –125 mmHg, is based on a limited study on pigs carried out in 1997 [17]. The use of one pressure in treating all wounds has been questioned by, among others, Kairinos *et al.* [128], and it has been suggested that the suction pressure should be adapted to suit the consistency and type of tissue, and the perfusion status of the wound. It is known from the present animal studies, and other studies by our group, that the maximal biological effects on the wound edges, in terms of wound contraction (Studies IV and V), regional blood flow (Study I) and the formation of granulation tissue [146], are obtained already at approximately –80 mmHg. Clinical studies have also shown that negative pressure levels of less than –125 mmHg result in excellent wound healing [147], and in a retrospective review on pediatric foam-based NPWT it was shown that the reduction in wound volume was unrelated to pressures between –50 and –125 mmHg [148]. When using NPWT for the treatment of poorly perfused tissue (e.g., diabetic foot ulcers and thin skin transplants), ischemia can develop at the wound edges. Pressures of higher magnitudes are also known to cause pain to the patient [56, 129]. Thus, it may be advantageous to reduce the negative pressure to, e.g., –40 mmHg in the treatment of sensitive, poorly perfused tissue, which is the level at which about half the maximal biological effect is seen (Studies I and V). There is seldom a reason to use a greater negative pressure than –80 mmHg, however, –125 mmHg could be used initially to improve the drainage of exudate in high-output wounds (Study V). A lower negative pressure can then be used for the further treatment of the wound.

A review of NPWT studies on the impact of the negative pressure level on the modes of action of NPWT was recently published [149]. The findings are presented in Figure 19 (below).
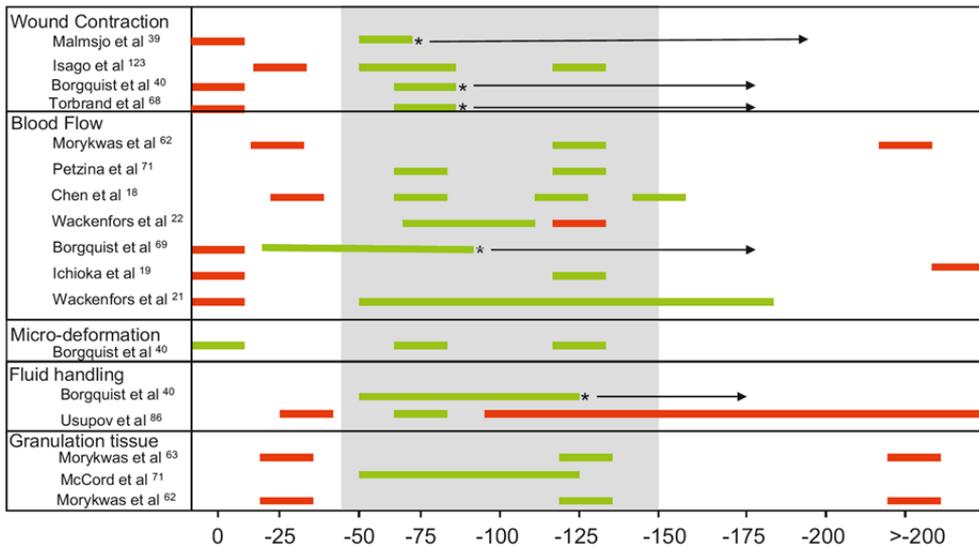
Figure 19. The impact of different levels of negative pressure (x-axis) on the modes of action of NPWT. Red bars indicate pressures at which no effect or detrimental effects have been observed. Green bars indicate pressures at which beneficial effects have been observed. The shaded area demonstrates the therapeutic range of negative pressure levels. (From [149].) * Although higher levels of negative pressure (denoted by arrows) may be effective, no further benefit is observed.

Thus, there is no single negative pressure level that is suitable for treating all wounds, but rather a range of pressures within which positive effects can be maximized and adverse effects hopefully avoided. The negative pressure level may be chosen based on the biological effects that are deemed most important for the wound in question.

Wound filler

The choice of wound filler material, foam or gauze, has considerable influence on the wound healing process, as seen by the amount and characteristics of granulation tissue. Therefore, this choice warrants careful consideration. Study IV showed that both foam- and gauze-based NPWT resulted in the same degree of micro- and macrodeformation. However, Wilkes *et al.* found that gauze produced only approximately 80% of the microdeformation produced by foam [64]. Studies in larger wounds have shown that macrodeformation is greater when using foam than gauze, presumably because of the open, porous texture of foam [150]. Furthermore, the degree of hypoperfusion in the superficial wound bed tissue has been reported to be greater when using foam than gauze [150]). Taken together, these findings can explain the clinical observation that foam is associated with more rapid surface granulation than gauze.

In case studies by Fraccalvieri *et al.*, hypertrophic granulation tissue was observed in the wound bed when using foam, whereas a thinner, denser and more uniform wound bed was seen with gauze-based NPWT [151]. Empirical evidence indicates that foam may be a good choice in wounds where large amounts of tissue are desirable (e.g., in sternotomy wounds). In acute wounds with large tissue loss, foam may offer a bridging therapy, causing the tissue to contract so that the wound edges are drawn together. When gauze is used, the newly formed tissue is thinner and more stable. Gauze has become a popular wound filler among plastic surgeons for wound-bed preparation before grafting. Empirical findings among clinicians, as well as animal studies, have shown that hypertrophic granulation tissue leads to fibrosis and scar tissue, as well as contractures; therefore, thick granulation tissue is undesirable when the cosmetic result is important, or in cases where scar tissue may restrict movement, e.g., over joints. In such cases, gauze is preferable. Another important factor that warrants consideration is the fact that foam shrinks when pressure is applied to it, which can be a mixed blessing. Although this is an advantage for wound healing, it is not desirable in wounds that have, or will need, a skin graft or a flap. It is also disadvantageous in wounds around a joint, where shrinkage causes contraction of the joint. A small case series of 18 NPWT-treated acute post-traumatic wounds revealed that newly reconstructed tissue in patients treated with gauze is more similar to physiological tissue than foam-treated tissue [151]. Ultrasonography showed that the thickness of scar tissue after treatment with foam is approximately twice that obtained after treatment with gauze [151].

Many wounds are highly irregular in shape and may extend over a large area, and over curved surfaces of the body. Foam can be difficult to cut into shape to fit such a wound, and there is a risk of small pieces of foam being lost in the wound. Gauze naturally assumes the contours of the wound bed [152], and can easily be cut to shape or packed into crevices, pockets, or tunnels. Large rolls of gauze are normally used,

and when inserted into deep structures, a longer "tail" must be left hanging into the main wound.

Ingrowth of the wound bed tissue into the wound filler is a recently discovered problem [153]. Contact between foam and the wound bed leads to ingrowth of tissue in the foam [146, 154], whereas no ingrowth is seen when gauze is used [146]. Therefore, more force is required to remove foam than gauze when changing dressings [146], which leads to pain. Also, some of the foam material may be left in the wound leading to non-selective mechanical debridement when it is removed. In a small, retrospective study of 27 pediatric patients treated with foam-based NPWT for various soft-tissue defects, the complications described were primarily related to bleeding caused by disruption of the granulation tissue during dressing changes [155]. Attempts have been made to reduce pain by applying local anesthetics to the surface of the wound [156], and it is not uncommon that morphine is administered systemically before dressing changes [157]. However, we believe that the use of local anesthetics is undesirable, as it may have a negative impact on wound healing [158]. According to recent recommendations regarding NPWT, gauze may be used in patients more susceptible to pain to reduce the pain experienced during dressing removal [32]. As foam- and gauze-based NPWT are both capable of delivering clinically relevant effects [149], the choice of wound filler may, at least partially, depend on practical considerations, such as ease of application, effects on patient acceptability/tolerance, availability and cost [149].

A wound contact layer, e.g., paraffin or silicon dressings, is sometimes placed between the wound bed and the wound filler to prevent ingrowth. In the clinical setting it is obvious that this reduces the pain when changing dressings, but it may also reduce the effect of the treatment on the wound bed, probably because these materials are semipermeable, and the pressure transmitted to the wound bed is reduced [159].

Continuous, intermittent and variable negative pressure

The most common mode of application of negative pressure is the continuous mode, i.e., a constant pressure. However, it has been shown that the amount of granulation tissue in the wound bed increases dramatically during intermittent NPWT [17], most probably as a result of the favorable combination of increasing and decreasing blood flow at the wound edges (which facilitates tissue oxygenation, angiogenesis and the removal of waste products), and the repeated mechanical stimulation of the wound edges. However, the sudden change in pressure causes the wound filler to expand and contract repeatedly over the granulation tissue, possibly causing pain to the patient [31]. Another problem associated with intermittent therapy can be maintaining the

seal, as the pressure is reduced to atmospheric pressure. When NPWT is used to stabilize a wound, e.g., over a skin graft, intermittent therapy obviously offers only an intermittent splinting effect.

Variable NPWT was introduced to provide a smoother transition between two different levels of negative pressure, to maintain the negative pressure environment throughout the therapy, and to minimize the problems of pain and sealing. Interestingly, it has been shown that the effects on macrodeformation, blood flow, and granulation tissue formation in the wound bed are similar with variable and intermittent NPWT. Variable NPWT thus seems to combine the advantages of continuous and intermittent therapies. When the advantages of intermittent therapy are needed, without the disadvantages of loss of seal as a result of return to atmospheric pressure, or loss of the splinting action of the dressing, variable NPWT could, therefore, be applied. We anticipate more use of variable NPWT in clinical care in the future.

Intermittent and variable NPWT may be advantageous when treating wounds at risk of developing ischemia, i.e., where the wound edge tissue is poorly vascularized. NPWT exerts pressure against the wound edge [122], which results in hypoperfusion. It is well known that the pressure level must sometimes be reduced during treatment with continuous NPWT to prevent ischemia. During intermittent and variable NPWT, pressure is alleviated repeatedly, which may prevent persistent tissue ischemia. Indeed, Holle *et al.* reported 100% tissue survival in seven random-pattern flaps with critical tip perfusion following intermittent NPWT at –100 mmHg [160].

It should be borne in mind that intermittent and, to some extent, variable NPWT may not be suitable for all types of wounds. Wounds or tissue that require stabilization, such as sternotomy wounds, tunneling, undermining, split-thickness skin grafts, venous ulcers, and fistulae, may best be treated with continuous NPWT [35, 161]. Furthermore, in highly exudating wounds a continuous suction force may be needed, especially initially, in order to ensure adequate drainage.

Closing remarks

NPWT has beyond doubt improved both wound healing and the quality of life for many patients since its commercial introduction in the 1990s. The optimum conditions for NPWT are still being debated, and it is likely that indication-specific protocols will be introduced in the future. The parameters of NPWT (the negative pressure level, the type of wound filler, and the mode of pressure application) can be tailored to the specific requirements of the wound. The research presented here can be regarded as a step in this direction. The present studies were carried out in pigs, and final wound healing effects were not evaluated. Clinical studies are needed to corroborate our findings and would provide much-needed guidance for providing optimal wound care.

Knowledge is of no value unless you put it into practice

Anton Chekhov

Acknowledgements

Att denna avhandling låtit sig skrivas beror till stor del på alla fantastiska människor i min omgivning. Jag vill därför tacka:

Malin Malmsjö, min huvudhandledare. Utan din kreativitet, din sprudlande energi, ditt inspirerande sätt, din organisationsförmåga och ditt positiva sinnelag hade antagligen samtliga delarbeten förblivit dammiga manuskript i min dator. Det har varit fantastiskt lärorikt att få arbeta tillsammans med dig! Kanske kan jag nu få tid att låna din isseglingsutrustning?

Richard Ingemansson, min bihandledare, för griskirurgiska stordåd och upplyftande diskussioner.

Lotta Gustafsson, som sammanförde mig med Malin – utan dig hade det inte blivit någon sårforskning.

Christian Torbrand, **Erik Anesäter**, **Erik Hedström**, **Sandra Lindstedt Ingemansson** och **Markus Roupé** – under de långdragna grisförsöksveckorna och det efterföljande manusskrivandet har det varit härligt att omges av ett sådant gäng! **Bodil Gesslein**, **Gisela Håkansson** och **Julia Waga** – ovärderliga hjälpredor i samband med grisförsöken.

Charles Lee, for "the thermodiffusion contribution" to Study II.

Helen Sheppard, som har gjort underverk med det engelska språket.

Bo Veisland, som har bistått med fantastiska illustrationer – dessvärre fick inte alla plats i kappan.

Tadeusz Wieloch, **Peter Reinstrup** och **Hans Friberg**, som jag haft förmånen att få ha som handledare och inspirationskällor under min tidiga forskarkarriär.

Kollegorna på anestesikliniken, som har stått för ett trevligt och stimulerande arbetsklimat de senaste åren. *Himlen är inte blå, den är grön*, som det står på väggen på operationsavdelningen i Malmö. Harvey MacKay lär för övrigt ha uttalat orden "Find something you love to do and you'll never work a day in your life" – vilket stämmer väl överens med min syn på anestesi- och intensivvårdsjobbet!

Anders, **Patrik** och **Pontus**, som har stått för (o)vetenskapliga diskussioner i tid och otid och dessutom bokstavligen räddat mitt liv en gång eller två. Ni har också försett mig med mat och kvinnlig fägring i form av **Lina**, **Michelle** och **Susanne**. **Andreas**

och **Stefan** har lärt mig att förlora i allehanda spel utan att ta illa upp. **Ullis** har stått för förnyandet av väsentliga delar av min garderob genom åren. **Hasse** har bejakat mina allra mest pojkaktiga intressen. **Kembro** har stått för de galnaste upptågen genom tiderna.

Lund, min favoritstad i vått och torrt – en akademisk bondby av storslagna mått! Så klart även **Barsebäckshamn**, som med fantastiska vyer och få störande moment gett god möjlighet att skriva i lugn och ro. **Bruce Springsteen** har sett till att kvällarna framför datorn bjudit på behaglig bakgrundsmusik.

Inga och **Hans**, ni är de bästa svärföräldrar man kan önska sig! Utan er hade vi saknat sådant som barnpassning, söndagsmiddagar, kafferep, värdefulla kommentarer till kappan, husbyggarhjälp och humoristiska inslag i mailboxen. Kort sagt: livet hade varit mycket krångligare utan er. Och tråkigare.

Rasmus och **Niklas**, mina bröder, som genom att uppfostra mig den hårda vägen har bidragit till att utveckla min skepsis och tävlingsinstinkt. Dessutom har ni utökat familjen Borgquist genom **Signe**, **Jennie** och alla underbara **syskonbarn**.

Mamma och **pappa**, mina favoritföräldrar alla kategorier. Hjälpsamma, omtänksamma, barnpassande, förebilder – ni är fantastiska! Tack för allt stöd!

Ebba och **Carl**, mina favvo-lekkamrater när skrivinspirationen tryter och barnsligheten tar överhand.

Maria. Stort tack för att du tolererat alla papper som legat utspridda i huset i tid och otid. Och för att du pysslat om kidsen medan jag skrivit. Jag älskar dig.

Don't be too optimistic. The light at the end of the tunnel could be a train.

Franz Kafka

References

1. Cohen I, Diegelmann R, Yager D, et al. Wound care and wound healing. 7th ed ed. New York: McGraw-Hill, 1999.
2. Lazarus GS, Cooper DM, Knighton DR, et al. Definitions and guidelines for assessment of wounds and evaluation of healing. Wound Repair Regen 1994; 2(3):165-70.
3. Singer AJ, Clark RA. Cutaneous wound healing. N Engl J Med 1999; 341(10):738-46.
4. Enoch S, Grey JE, Harding KG. Recent advances and emerging treatments. BMJ 2006; 332(7547):962-5.
5. Gordillo GM, Sen CK. Revisiting the essential role of oxygen in wound healing. Am J Surg 2003; 186(3):259-63.
6. Gaffney EA, Pugh K, Maini PK, Arnold F. Investigating a simple model of cutaneous wound healing angiogenesis. J Math Biol 2002; 45(4):337-74.
7. Bollero D, Driver V, Glat P, et al. The Role of Negative Pressure Wound Therapy in the Spectrum of Wound Healing. Ostomy Wound Manage 2010; 56(5 Suppl):1-18.
8. Mendez-Eastman S. Guidelines for using negative pressure wound therapy. Adv Skin Wound Care 2001; 14(6):314-22; quiz 324-5.
9. Raffl AB. The use of negative pressure under skin flaps after radical mastectomy. Ann Surg 1952; 136(6):1048.
10. Miller MS, Lowery CA. Negative pressure wound therapy: "a rose by any other name". Ostomy Wound Manage 2005; 51(3):44-6, 48-9.
11. Kostiuchenok BM, Kolker, II, Karlov VA, et al. [Vacuum treatment in the surgical management of suppurative wounds]. Vestn Khir Im I I Grek 1986; 137(9):18-21.
12. Usupov Y, Yepifanov M. [Active wound drainage]. Vestnik khirugii 1987; 4:42-5.
13. Chariker M, Jeter K, Tintle T. Effective management of incisional and cutaneous fistulae with closed suction wound drainage. Contemp Surg 1989; 34:59-63.
14. Fleischmann W, Lang E, Russ M. [Treatment of infection by vacuum sealing]. Unfallchirurg 1997; 100(4):301-4.
15. Fleischmann W, Russ M, Westhauser A, Stampehl M. [Vacuum sealing as carrier system for controlled local drug administration in wound infection]. Unfallchirurg 1998; 101(8):649-54.
16. Fleischmann W, Strecker W, Bombelli M, Kinzl L. [Vacuum sealing as treatment of soft tissue damage in open fractures]. Unfallchirurg 1993; 96(9):488-92.

17. Morykwas MJ, Argenta LC, Shelton-Brown EI, McGuirt W. Vacuum-assisted closure: a new method for wound control and treatment: animal studies and basic foundation. Ann Plast Surg 1997; 38(6):553-62.

18. Shweiki E, Gallagher KE. Negative pressure wound therapy in acute, contaminated wounds: documenting its safety and efficacy to support current global practice. Int Wound J 2013; 10(1):13-43.

19. Stannard JP, Volgas DA, McGwin G, 3rd, et al. Incisional negative pressure wound therapy after high-risk lower extremity fractures. J Orthop Trauma 2012; 26(1):37-42.

20. Wanner MB, Schwarzl F, Strub B, et al. Vacuum-assisted wound closure for cheaper and more comfortable healing of pressure sores: a prospective study. Scand J Plast Reconstr Surg Hand Surg 2003; 37(1):28-33.

21. Ford CN, Reinhard ER, Yeh D, et al. Interim analysis of a prospective, randomized trial of vacuum-assisted closure versus the healthpoint system in the management of pressure ulcers. Ann Plast Surg 2002; 49(1):55-61; discussion 61.

22. Baharestani MM, Houliston-Otto DB, Barnes S. Early versus late initiation of negative pressure wound therapy: examining the impact on home care length of stay. Ostomy Wound Manage 2008; 54(11):48-53.

23. Armstrong DG, Lavery LA. Negative pressure wound therapy after partial diabetic foot amputation: a multicentre, randomised controlled trial. Lancet 2005; 366(9498):1704-10.

24. Blume PA, Walters J, Payne W, et al. Comparison of negative pressure wound therapy using vacuum-assisted closure with advanced moist wound therapy in the treatment of diabetic foot ulcers: a multicenter randomized controlled trial. Diabetes Care 2008; 31(4):631-6.

25. McCallon SK, Knight CA, Valiulus JP, et al. Vacuum-assisted closure versus saline-moistened gauze in the healing of postoperative diabetic foot wounds. Ostomy Wound Manage 2000; 46(8):28-32, 34.

26. Moues CM, van den Bemd GJ, Meerding WJ, Hovius SE. An economic evaluation of the use of TNP on full-thickness wounds. J Wound Care 2005; 14(5):224-7.

27. Braakenburg A, Obdeijn MC, Feitz R, et al. The clinical efficacy and cost effectiveness of the vacuum-assisted closure technique in the management of acute and chronic wounds: a randomized controlled trial. Plast Reconstr Surg 2006; 118(2):390-7; discussion 398-400.

28. Schwien T, Gilbert J, Lang C. Pressure ulcer prevalence and the role of negative pressure wound therapy in home health quality outcomes. Ostomy Wound Manage 2005; 51(9):47-60.

29. Llanos S, Danilla S, Barraza C, et al. Effectiveness of negative pressure closure in the integration of split thickness skin grafts: a randomized, double-masked, controlled trial. Ann Surg 2006; 244(5):700-5.

30. Vuerstaek JD, Vainas T, Wuite J, et al. State-of-the-art treatment of chronic leg ulcers: A randomized controlled trial comparing vacuum-assisted closure (V.A.C.) with modern wound dressings. J Vasc Surg 2006; 44(5):1029-37; discussion 1038.

31. Gupta S, Baharestani M, Baranoski S, et al. Guidelines for managing pressure ulcers with negative pressure wound therapy. Adv Skin Wound Care 2004; 17 Suppl 2:1-16.

32. Vig S, Dowsett C, Berg L, et al. Evidence-based recommendations for the use of negative pressure wound therapy in chronic wounds: steps towards an international consensus. J Tissue Viability 2011; 20 Suppl 1:S1-18.

33. Orgill DP, Manders EK, Sumpio BE, et al. The mechanisms of action of vacuum assisted closure: more to learn. Surgery 2009; 146(1):40-51.

34. Goldstein JA, Iorio ML, Brown B, Attinger CE. The use of negative pressure wound therapy for random local flaps at the ankle region. J Foot Ankle Surg 2010; 49(6):513-6.

35. Malmsjo M, Ingemansson R, Sjogren J. Mechanisms governing the effects of vacuum-assisted closure in cardiac surgery. Plast Reconstr Surg 2007; 120(5):1266-75.

36. Wackenfors A, Sjogren J, Gustafsson R, et al. Effects of vacuum-assisted closure therapy on inguinal wound edge microvascular blood flow. Wound Repair Regen 2004; 12(6):600-6.

37. Wackenfors A, Gustafsson R, Sjogren J, et al. Blood flow responses in the peristernal thoracic wall during vacuum-assisted closure therapy. Ann Thorac Surg 2005; 79(5):1724-30; discussion 1730-1.

38. Lindstedt S, Malmsjo M, Gesslein B, Ingemansson R. Topical negative pressure effects on coronary blood flow in a sternal wound model. Int Wound J 2008; 5(4):503-9.

39. Lindstedt S, Malmsjo M, Ingemansson R. No hypoperfusion is produced in the epicardium during application of myocardial topical negative pressure in a porcine model. J Cardiothorac Surg 2007; 2:53.

40. Lindstedt S, Malmsjo M, Ingemansson R. Blood flow changes in normal and ischemic myocardium during topically applied negative pressure. Ann Thorac Surg 2007; 84(2):568-73.

41. Petrie N, Potter M, Banwell P. The management of lower extremity wounds using topical negative pressure. Int J Low Extrem Wounds 2003; 2(4):198-206.

42. Banwell PE. Topical negative pressure therapy in wound care. J Wound Care 1999; 8(2):79-84.

43. Argenta LC, Morykwas MJ. Vacuum-assisted closure: a new method for wound control and treatment: clinical experience. Ann Plast Surg 1997; 38(6):563-76; discussion 577.

44. Morykwas MJ, Simpson J, Punger K, et al. Vacuum-assisted closure: state of basic research and physiologic foundation. Plast Reconstr Surg 2006; 117(7 Suppl):121S-126S.

45. Stannard JP, Robinson JT, Anderson ER, et al. Negative pressure wound therapy to treat hematomas and surgical incisions following high-energy trauma. J Trauma 2006; 60(6):1301-6.

46. Eginton MT, Brown KR, Seabrook GR, et al. A prospective randomized evaluation of negative-pressure wound dressings for diabetic foot wounds. Ann Vasc Surg 2003; 17(6):645-9.

47. Joseph E, Hamori CA, Bergman S, et al. A prospective randomized trial of vacuum-assisted closure versus standard therapy of chronic nonhealing wounds. Wounds 2000; 12(3):60-7.

48. Morykwas MJ, Faler BJ, Pearce DJ, Argenta LC. Effects of varying levels of subatmospheric pressure on the rate of granulation tissue formation in experimental wounds in swine. Ann Plast Surg 2001; 47(5):547-51.

49. Moues CM, van den Bemd GJ, Heule F, Hovius SE. Comparing conventional gauze therapy to vacuum-assisted closure wound therapy: a prospective randomised trial. J Plast Reconstr Aesthet Surg 2007; 60(6):672-81.

50. Malmsjo M, Ingemansson R, Martin R, Huddleston E. Negative-pressure wound therapy using gauze or open-cell polyurethane foam: similar early effects on pressure transduction and tissue contraction in an experimental porcine wound model. Wound Repair Regen 2009; 17(2):200-5.

51. Saxena V, Hwang CW, Huang S, et al. Vacuum-assisted closure: microdeformations of wounds and cell proliferation. Plast Reconstr Surg 2004; 114(5):1086-96; discussion 1097-8.

52. Kairinos N, Voogd AM, Botha PH, et al. Negative-pressure wound therapy II: negative-pressure wound therapy and increased perfusion. Just an illusion? Plast Reconstr Surg 2009; 123(2):601-12.

53. Chen SZ, Li J, Li XY, Xu LS. Effects of vacuum-assisted closure on wound microcirculation: an experimental study. Asian J Surg 2005; 28(3):211-7.

54. Greene AK, Puder M, Roy R, et al. Microdeformational wound therapy: effects on angiogenesis and matrix metalloproteinases in chronic wounds of 3 debilitated patients. Ann Plast Surg 2006; 56(4):418-22.

55. Ichioka S, Shibata M, Kosaki K, et al. Effects of shear stress on wound-healing angiogenesis in the rabbit ear chamber. J Surg Res 1997; 72(1):29-35.

56. Timmers MS, Le Cessie S, Banwell P, Jukema GN. The effects of varying degrees of pressure delivered by negative-pressure wound therapy on skin perfusion. Ann Plast Surg 2005; 55(6):665-71.

57. Nease C. Using low pressure, negative pressure wound therapy for wound preparation and the management of split-thickness skin grafts in three patients with complex wounds. Ostomy Wound Management 2009; 55(6):32-42.

58. Ahearn C. Intermittent NPWT and lower negative pressures--exploring the disparity between science and current practice: a review. Ostomy Wound Manage 2009; 55(6):22-8.

59. Ichioka S, Watanabe H, Sekiya N, et al. A technique to visualize wound bed microcirculation and the acute effect of negative pressure. Wound Repair Regen 2008; 16(3):460-5.

60. Malmsjo M, Ingemansson R, Lindstedt S, Gustafsson L. Comparison of bacteria and fungus-binding mesh, foam and gauze as fillers in negative pressure wound therapy - pressure transduction, wound edge contraction, microvascular blood flow and fluid retention. Int Wound J [Epub ahead of print].

61. Lavery LA, Barnes SA, Keith MS, et al. Prediction of healing for postoperative diabetic foot wounds based on early wound area progression. Diabetes Care 2008; 31(1):26-9.

62. Dunn RM, Ignotz R, Mole T, et al. Assessment of gauze-based negative pressure wound therapy in the split-thickness skin graft clinical pathway-an observational study. Eplasty 2011; 11:e14.

63. Bassetto F, Lancerotto L, Salmaso R, et al. Histological evolution of chronic wounds under negative pressure therapy. J Plast Reconstr Aesthet Surg 2012; 65(1):91-9.

64. Wilkes R, Zhao Y, Kieswetter K, Haridas B. Effects of Dressing Type on 3D Tissue Microdeformations During Negative Pressure Wound Therapy: A Computational Study. J Biomech Eng 2009; 131(3):031012.

65. McNulty A, Spranger I, Courage J, et al. The consistent delivery of negative pressure to wounds using reticulated open cell foam and regulated pressure feedback. Wounds 2010; 22(5):114-20.

66. 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(3):272-87.

67. Heit YI, Dastouri P, Helm DL, et al. Foam pore size is a critical interface parameter of suction-based wound healing devices. Plast Reconstr Surg 2012; 129(3):589-97.

68. Huang S, Chen CS, Ingber DE. Control of cyclin D1, p27(Kip1), and cell cycle progression in human capillary endothelial cells by cell shape and cytoskeletal tension. Mol Biol Cell 1998; 9(11):3179-93.

69. Chen CS, Mrksich M, Huang S, et al. Geometric control of cell life and death. Science 1997; 276(5317):1425-8.

70. Malmsjo M, Ingemansson R. Similar biological effects of green and black polyurethane foam in negative pressure wound therapy: green foam facilitates monitoring of wound status, bleeding and exudate. 20th Conference of the European Wound Management Association. Geneva, Switzerland, 2010.

71. Scherer SS, Pietramaggiori G, Mathews JC, et al. The mechanism of action of the vacuum-assisted closure device. Plast Reconstr Surg 2008; 122(3):786-97.

72. Huang S, Ingber DE. Shape-dependent control of cell growth, differentiation, and apoptosis: switching between attractors in cell regulatory networks. Exp Cell Res 2000; 261(1):91-103.

73. Chen CS, Mrksich M, Huang S, et al. Micropatterned surfaces for control of cell shape, position, and function. Biotechnol Prog 1998; 14(3):356-63.

74. Li J, Hampton T, Morgan JP, Simons M. Stretch-induced VEGF expression in the heart. J Clin Invest 1997; 100(1):18-24.

75. Seko Y, Takahashi N, Shibuya M, Yazaki Y. Pulsatile stretch stimulates vascular endothelial growth factor (VEGF) secretion by cultured rat cardiac myocytes. Biochem Biophys Res Commun 1999; 254(2):462-5.

76. Chang H, Wang BW, Kuan P, Shyu KG. Cyclical mechanical stretch enhances angiopoietin-2 and Tie2 receptor expression in cultured human umbilical vein endothelial cells. Clin Sci (Lond) 2003; 104(4):421-8.

77. Cloutier M, Maltais F, Piedboeuf B. Increased distension stimulates distal capillary growth as well as expression of specific angiogenesis genes in fetal mouse lungs. Exp Lung Res 2008; 34(3):101-13.

78. Labler L, Rancan M, Mica L, et al. Vacuum-assisted closure therapy increases local interleukin-8 and vascular endothelial growth factor levels in traumatic wounds. J Trauma 2009; 66(3):749-57.

79. Shiu YT, Weiss JA, Hoying JB, et al. The role of mechanical stresses in angiogenesis. Crit Rev Biomed Eng 2005; 33(5):431-510.

80. Ingber DE, Prusty D, Sun Z, et al. Cell shape, cytoskeletal mechanics, and cell cycle control in angiogenesis. J Biomech 1995; 28(12):1471-84.

81. Von Offenberg Sweeney N, Cummins PM, Cotter EJ, et al. Cyclic strain-mediated regulation of vascular endothelial cell migration and tube formation. Biochem Biophys Res Commun 2005; 329(2):573-82.

82. Folkman J, Moscona A. Role of cell shape in growth control. Nature 1978; 273(5661):345-9.

83. Molnar JA. The science behind negative pressure wound therapy. Ostomy Wound Manage 2004; 50(4A Suppl):2-5.

84. Moues CM, van Toorenenbergen AW, Heule F, et al. The role of topical negative pressure in wound repair: expression of biochemical markers in wound fluid during wound healing. Wound Repair Regen 2008; 16(4):488-94.

85. Okan D, Woo K, Ayello EA, Sibbald G. The role of moisture balance in wound healing. Adv Skin Wound Care 2007; 20(1):39-53; quiz 53-5.

86. Bishop SM, Walker M, Rogers AA, Chen WY. Importance of moisture balance at the wound-dressing interface. J Wound Care 2003; 12(4):125-8.

87. Kerstein MD. Moist wound healing: the clinical perspective. Ostomy Wound Manage 1995; 41(7A Suppl):37S-44S; discussion 45S.

88. Hinman CD, Maibach H. Effect of Air Exposure and Occlusion on Experimental Human Skin Wounds. Nature 1963; 200:377-8.

89. Alvarez OM, Mertz PM, Eaglstein WH. The effect of occlusive dressings on collagen synthesis and re-epithelialization in superficial wounds. J Surg Res 1983; 35(2):142-8.

90. Hien NT, Prawer SE, Katz HI. Facilitated wound healing using transparent film dressing following Mohs micrographic surgery. Arch Dermatol 1988; 124(6):903-6.

91. Pirone LA, Monte KA, Shannon RJ, Bolton LL. Wound healing under occlusion and non-occlusion in partial-thickness and full-thickness wounds in swine. Wounds 1990; 2(2):74-81.

92.   Caruso DM, Foster KN, Blome-Eberwein SA, et al. Randomized clinical study of Hydrofiber dressing with silver or silver sulfadiazine in the management of partial-thickness burns. J Burn Care Res 2006; 27(3):298-309.

93.   Madden MR, Nolan E, Finkelstein JL, et al. Comparison of an occlusive and a semi-occlusive dressing and the effect of the wound exudate upon keratinocyte proliferation. J Trauma 1989; 29(7):924-30; discussion 930-1.

94.   Lohsiriwat V, Chuangsuwanich A. Comparison of the ionic silver-containing hydrofiber and paraffin gauze dressing on split-thickness skin graft donor sites. Ann Plast Surg 2009; 62(4):421-2.

95.   Kunugiza Y, Tomita T, Moritomo H, Yoshikawa H. A hydrocellular foam dressing versus gauze: effects on the healing of rat excisional wounds. J Wound Care 2010; 19(1):10-4.

96.   Andriessen A, Polignano R, Abel M. Monitoring the microcirculation to evaluate dressing performance in patients with venous leg ulcers. J Wound Care 2009; 18(4):145-50.

97.   Chen WY, Rogers AA, Lydon MJ. Characterization of biologic properties of wound fluid collected during early stages of wound healing. J Invest Dermatol 1992; 99(5):559-64.

98.   Leipziger LS, Glushko V, DiBernardo B, et al. Dermal wound repair: role of collagen matrix implants and synthetic polymer dressings. J Am Acad Dermatol 1985; 12(2 Pt 2):409-19.

99.   Schultz GS, Sibbald RG, Falanga V, et al. Wound bed preparation: a systematic approach to wound management. Wound Repair Regen 2003; 11 Suppl 1:S1-28.

100. Grinnell F, Ho CH, Wysocki A. Degradation of fibronectin and vitronectin in chronic wound fluid: analysis by cell blotting, immunoblotting, and cell adhesion assays. J Invest Dermatol 1992; 98(4):410-6.

101. Trengove NJ, Bielefeldt-Ohmann H, Stacey MC. Mitogenic activity and cytokine levels in non-healing and healing chronic leg ulcers. Wound Repair Regen 2000; 8(1):13-25.

102. Wysocki AB. Wound fluids and the pathogenesis of chronic wounds. J Wound Ostomy Continence Nurs 1996; 23(6):283-90.

103. Stechmiller JK, Kilpadi DV, Childress B, Schultz GS. Effect of Vacuum-Assisted Closure Therapy on the expression of cytokines and proteases in wound fluid of adults with pressure ulcers. Wound Repair Regen 2006; 14(3):371-4.

104. Miller MS. Commentary: New Microvascular Blood Flow Research Challenges Practice Protocols in Negative Pressure Wound Therapy. 2005; 17(Oct):290-4.

105. Gasbarro R. Negative pressure wound therapy: a clinical review. Wounds 2007; 19(12):1-7.

106. Willy C, von Thun-Hohenstein H, von Lubken F, et al. [Experimental principles of the V.A.C.-therapy -- pressure values in superficial soft tissue and the applied foam]. Zentralbl Chir 2006; 131 Suppl 1:S50-61.

107. Maier D, Beck A, Kinzl L, Bischoff M. [The physics of vacuum therapy]. Zentralbl Chir 2005; 130(5):463-8.

108. Murphey GC, Macias BR, Hargens AR. Depth of penetration of negative pressure wound therapy into underlying tissues. Wound Repair Regen 2009; 17(1):113-7.
109. Malmsjo M, Ingemansson R, Martin R, Huddleston E. Wound edge microvascular blood flow: effects of negative pressure wound therapy using gauze or polyurethane foam. Ann Plast Surg 2009; 63(6):676-81.
110. Petzina R, Gustafsson L, Mokhtari A, et al. Effect of vacuum-assisted closure on blood flow in the peristernal thoracic wall after internal mammary artery harvesting. Eur J Cardiothorac Surg 2006; 30(1):85-9.
111. Kamolz LP, Andel H, Haslik W, et al. Use of subatmospheric pressure therapy to prevent burn wound progression in human: first experiences. Burns 2004; 30(3):253-8.
112. Glass GE, Nanchahal J. The methodology of negative pressure wound therapy: separating fact from fiction. J Plast Reconstr Aesthet Surg 2012; 65(8):989-1001.
113. Argenta LC, Morykwas MJ, Marks MW, et al. Vacuum-assisted closure: state of clinic art. Plast Reconstr Surg 2006; 117(7 Suppl):127S-142S.
114. Fraccalvieri M, Ruka E, Bocchiotti MA, et al. Patient's pain feedback using negative pressure wound therapy with foam and gauze. Int Wound J 2011; 8(5):492-9.
115. Hurd T, Chadwick P, Cote J, et al. Impact of gauze-based NPWT on the patient and nursing experience in the treatment of challenging wounds. Int Wound J 2010; 7(6):448-55.
116. Jeffery SL. Advanced wound therapies in the management of severe military lower limb trauma: a new perspective. Eplasty 2009; 9:e28.
117. Malmsjö M, Ingemansson R. Variable, intermittent and continuous negative pressure wound therapy using foam or gauze - the biological effects on the wound bed including, blood flow, micro and macrodeformation, granulation tissue quantity, wound bed characteristics and tissue ingrowth into the wound filler. Abstract at the Symposium on Advanced Wound Care and the Wound Healing Society Meeting in Orlando, Florida. 2010; April 17-20.
118. Eberlein T, Fendler H. Using a new technique of negative pressure wound therapy (NPWT), variable pressure therapy (VPT), for the management of chronic, non-healing wounds. Clinical Symposium for Advanced Wound Care (SAWC; Dallas, Texas) 2009; April.
119. Sullivan TP, Eaglstein WH, Davis SC, Mertz P. The pig as a model for human wound healing. Wound Repair Regen 2001; 9(2):66-76.
120. Zografos GC, Martis K, Morris DL. Laser Doppler flowmetry in evaluation of cutaneous wound blood flow using various suturing techniques. Ann Surg 1992; 215(3):266-8.
121. Goode TL, Klein HJ. Miniaturization: an overview of biotechnologies for monitoring the physiology and pathophysiology of rodent animal models. ILAR J 2002; 43(3):136-46.
122. Kairinos N, Solomons M, Hudson DA. The paradox of negative pressure wound therapy--in vitro studies. J Plast Reconstr Aesthet Surg 2010; 63(1):174-9.

123. Ichioka S, Watanabe H, Sekiya N, et al. A technique to visualize wound bed microcirculation and the acute effect of negative pressure. Wound Rep Reg 2008; 16:460-465.

124. Jonsson K, Jensen JA, Goodson WHI, et al. Tissue oxygenation, anemia, and perfusion in relation to wound healing in surgical patients. Ann Surg 1991; 214(5):605-13.

125. Willy C, Gerngross H. [Scientific background of the vacuum closure - an abstract]. Zentralbl Chir 2004; 129 Suppl 1:S6.

126. Venturi ML, Attinger CE, Mesbahi AN, et al. Mechanisms and clinical applications of the vacuum-assisted closure (VAC) Device: a review. Am J Clin Dermatol 2005; 6(3):185-94.

127. Attinger CE, Janis JE, Steinberg J, et al. Clinical approach to wounds: debridement and wound bed preparation including the use of dressings and wound-healing adjuvants. Plast Reconstr Surg 2006; 117(7 Suppl):72S-109S.

128. Kairinos N, Solomons M, Hudson DA. Negative-pressure wound therapy I: the paradox of negative-pressure wound therapy. Plast Reconstr Surg 2009; 123(2):589-98; discussion 599-600.

129. Krasner DL. Managing wound pain in patients with vacuum-assisted closure devices. Ostomy Wound Manage 2002; 48(5):38-43.

130. Malmsjö M, Ingemansson R, Martin R, Huddleston E. Negative-pressure wound therapy using gauze or open-cell polyurethane foam: similar early effects on pressure transduction and tissue contraction in an experimental porcine wound model. Wound Repair Regen 2009; 17(2):200-5.

131. Etöz A ÖY, Özcan M. The use of negative pressure wound therapy on diabetic foot ulcers: a preliminary controlled trial. Wounds 2004(16):264-9.

132. Campbell PE, Smith GS, Smith JM. Retrospective clinical evaluation of gauze-based negative pressure wound therapy. Int Wound J 2008; 5(2):280-6.

133. Malmsjo M, Lindstedt S, Ingemansson R. Effects of foam or gauze on sternum wound contraction, distension and heart and lung damage during negative-pressure wound therapy of porcine sternotomy wounds. Interact Cardiovasc Thorac Surg 2011; 12(3):349-54.

134. Etöz A, Özgenel Y, Özcan M. The use of negative pressure wound therapy on diabetic foot ulcers: a preliminary controlled trial. Wounds 2004(16):264-9.

135. Isago T, Nozaki M, Kikuchi Y, et al. Effects of different negative pressures on reduction of wounds in negative pressure dressings. J Dermatol 2003; 30(8):596-601.

136. Silver FH, Siperko LM. Mechanosensing and mechanochemical transduction: how is mechanical energy sensed and converted into chemical energy in an extracellular matrix? Crit Rev Biomed Eng 2003; 31(4):255-331.

137. Lindstedt S, Malmsjo M, Hansson J, et al. Pressure transduction and fluid evacuation during conventional negative pressure wound therapy of the open abdomen and NPWT using a protective disc over the intestines. BMC Surg 2012; 12:4.

138. Usupov YN, Yepifanov MV. Active Wound Drainage. Vestnik Khirurgii 1987:42-45.

139. Bucalo B, Eaglstein WH, Falanga V. Inhibition of cell proliferation by chronic wound fluid. Wound Repair Regen 1993; 1(3):181-6.

140. Grinnell F, Zhu M. Identification of neutrophil elastase as the proteinase in burn wound fluid responsible for degradation of fibronectin. J Invest Dermatol 1994; 103(2):155-61.

141. Mast BA, Schultz GS. Interactions of cytokines, growth factors, and proteases in acute and chronic wounds. Wound Repair Regen 1996; 4(4):411-20.

142. Wysocki AB, Staiano-Coico L, Grinnell F. Wound fluid from chronic leg ulcers contains elevated levels of metalloproteinases MMP-2 and MMP-9. J Invest Dermatol 1993; 101(1):64-8.

143. Chen SM, Ward SI, Olutoye OO, et al. Ability of chronic wound fluids to degrade peptide growth factors is associated with increased levels of elastase activity and diminished levels of proteinase inhibitors. Wound Repair Regen 1997; 5(1):23-32.

144. Ladwig GP, Robson MC, Liu R, et al. Ratios of activated matrix metalloproteinase-9 to tissue inhibitor of matrix metalloproteinase-1 in wound fluids are inversely correlated with healing of pressure ulcers. Wound Repair Regen 2002; 10(1):26-37.

145. Tarlton JF, Bailey AJ, Crawford E, et al. Prognostic value of markers of collagen remodeling in venous ulcers. Wound Repair Regen 1999; 7(5):347-55.

146. Borgquist O, Gustafsson L, Ingemansson R, Malmsjo M. Tissue ingrowth into foam but not into gauze during negative pressure wound therapy. Wounds 2009; 21(11):302-9.

147. Nease C. Using low pressure, negative pressure wound therapy for wound preparation and the management of split-thickness skin grafts in three patients with complex wounds. Ostomy Wound Manage 2009; 55(6):32-42.

148. McCord SS, Naik-Mathuria BJ, Murphy KM, et al. Negative pressure therapy is effective to manage a variety of wounds in infants and children. Wound Repair Regen 2007; 15(3):296-301.

149. Birke-Sorensen H, Malmsjo M, Rome P, et al. Evidence-based recommendations for negative pressure wound therapy: treatment variables (pressure levels, wound filler and contact layer)--steps towards an international consensus. J Plast Reconstr Aesthet Surg 2011; 64 Suppl:S1-16.

150. Malmsjo M, Ingemansson R. Effects of green foam, black foam and gauze on contraction, blood flow and pressure delivery to the wound bed in negative pressure wound therapy. J Plast Reconstr Aesthet Surg 2011; 64(12):e289-96.

151. Fraccalvieri M, Zingarelli E, Ruka E, et al. Negative pressure wound therapy using gauze and foam: histological, immunohistochemical and ultrasonography morphological analysis of the granulation tissue and scar tissue. Preliminary report of a clinical study. Int Wound J 2011; 8(4):355-64.

152. Taylor CJ, Chester DL, Jeffery SL. Functional splinting of upper limb injuries with gauze-based topical negative pressure wound therapy. J Hand Surg Am 2011; 36(11):1848-51.

153. Administration USFaD. FDA Safety Communication: UPDATE on Serious Complications Associated with Negative Pressure Wound Therapy Systems. 2011.

154. Morykwas M. Sub-Atmospheric Pressure Therapy: Research Evidence. 1st International Topical Negative Pressure Therapy ETRS Focus Group Meeting. London: ETRS, 2003. pp. 39-44.

155. Mooney JF, 3rd, Argenta LC, Marks MW, et al. Treatment of soft tissue defects in pediatric patients using the V.A.C. system. Clin Orthop Relat Res 2000(376):26-31.

156. Franczyk M, Lohman RF, Agarwal JP, et al. The impact of topical lidocaine on pain level assessment during and after vacuum-assisted closure dressing changes: a double-blind, prospective, randomized study. Plast Reconstr Surg 2009; 124(3):854-61.

157. Ahearn C. Intermittent Negative Pressure Wound Therapy and Lower Negative Pressures - Exploring the Disparity between Science and Current Practice: A Review of the Literature. Ostomy Wound Manage 2009; 55(6):22-8.

158. Zeren S, Kesici S, Kesici U, et al. Technical communication: effects of levobupivacaine on wound healing. Anesth Analg 2013; 116(2):495-9.

159. Jones SM, Banwell PE, Shakespeare PG. Interface dressings influence the delivery of topical negative-pressure therapy. Plast Reconstr Surg 2005; 116(4):1023-8.

160. Holle G, Peek A, Fritschen v U, Exner K. Von der komplizierten Wunde zum Tissue-engineering. Besondere Indikationen für die V. A. C. Therapie. Jahrestagung Vereinigung der Deutschen Plastischer Chirurgen 2002 (Abstractband 33).

161. Bovill E, Banwell PE, Teot L, et al. Topical negative pressure wound therapy: a review of its role and guidelines for its use in the management of acute wounds. Int Wound J 2008; 5(4):511-29.