NEGATIVE PRESSURE WOUND HEALING: HELP OR HASSLE?

By

Kathryn A. Pitt

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Comparative Medicine and Integrative Biology

2012

ABSTRACT

NEGATIVE PRESSURE WOUND HEALING: HELP OR HASSLE?

By

Kathryn A. Pitt

Negative pressure wound therapy (NPWT), is widely used in the human wound care setting, most commonly for acute traumas, dehiscences, and free grafts. NPWT is a mechanical adjunct that has shown to be effective in the treatment of these wounds. The therapy is thought to decrease interstitial edema, increase perfusion to the wound and periwound, and the mechanical strain on the fibroblasts appears to encourage fibroplasia. Its use in veterinary medicine is promising, with one of the advantages being prolonged time between dressing changes (up to 72 hours). At the Michigan State University College of Veterinary Medicine we have used NPWT in over 70 patients in the Veterinary Teaching Hospital, and conducted two controlled studies showing positive effects of NPWT on open wound healing and acceptance of full-thickness skin grafts.

This thesis details wound healing and open wound therapies, the putative mechanisms of action of NPWT, and its clinical applications (Chapter 1). A prospective feasibility study of 50 cases of NPWT is also described (Chapter 2), as well as the manuscript of a controlled experimental study investigating the effects of NPWT on skin graft acceptance (Chapter 3). Further studies and directions for the Michigan State Wound Healing and Management Laboratory are also discussed (Chapter 4).

ACKNOWLEDGEMENTS

I would like to thank Dr. Bryden Stanley for all of her support and guidance during my Masters year as well as during my time as a DVM student. Her knowledge, enthusiasm, and perseverance are inspiring. I hope to continue learning from her and having her as a mentor throughout my career.

I would also like to thank Kristin Aybar, LVT for helping me with the coordination, organization, and execution of this project.

Additionally, I would like to thank all of the veterinary technicians that work in the Nursing Care Unit of the Veterinary Teaching Hospital for their help in caring for our patients while in-hospital.

I would also like to thank Michele Fritz, LVT for all of her help and support in past projects in addition to the current project.

Many thanks to Dr. Joe Hauptman, Dr. Sue Holcombe, and Dr. Barb Steficek for all of their support and guidance as members of my Masters guidance committee.

Finally, I would like to thank my friends and family. Their unconditional support has helped me to reach where I am today. Without them, I would not be trying to reach my full potential, and would have become a beach bum a long time ago.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

KEY TO SYMBOLS OR ABBREVIATIONS

MSU VTH – Michigan State University Veterinary Teaching Hospital

NPWT – Negative pressure wound therapy

INPWT – Incisional negative pressure wound therapy

PDGF - Platelet-derived growth factor

TGF-β - Transforming growth factor

EGF - Epidermal growth factor

VEGF - Vascular endothelial growth factor

FGF - Fibroblast growth factor

bFGF - Basic fibroblast growth factor

TNF-α - Tumor necrosis factor-α

MMP - Matrix metalloproteinase

PRP – Platelet Rich Plasma

HAIS – Histologic acute inflammation score

CON – Control

H&E - Hemotoxylin and eosin

FTSG – Full thickness skin graft

ANOVA – Analysis of variance

WHAM – Wound healing and management laboratory

CHAPTER 1: Wound Healing and Topical Therapies

INTRODUCTION

The care of wounds has evolved greatly over the past century, largely due to the understanding of wound infection and the development of antibacterial practices and drugs. There have been even more advances in wound management in the last twenty years, because of our improved understanding of the cellular and subcellular processes of wound healing. In 2003, human wound care costs in the United States alone were estimated to be more than $20 billion, and of that, more than $6 billion was spent for the treatment of diabetic foot ulcers.[1-4]

Continuous advancements in the understanding of wound healing and its processes have been made. With the development of technology we have been able to study cell types, growth factors, enzymes, collagen, fibroblasts, and the order in which they appear in a healing wound. All types of wounds from a controlled incision made by a scalpel blade, to injuries from the trauma of a car crash or bullet wound, result in the same reparative process.

HISTORY OF WOUND HEALING

The response of tissue to injury and the wound healing processes are similar in all mammals, thus study of both human and veterinary wound management can benefit both disciplines. Wound healing´s predictable sequence of events has allowed our approach to it evolve throughout the centuries. Documentation of wound healing and medicine dates back to the Ancient Egyptians.[5,6] The Edwin Smith Surgical Papyrus is a document, translated from its original hieroglyphics, which details 48 cases of wounds with a rational and scientific approach (**Figure 1.1;** all tables and figures can be found in the appendices following their respective chapter).[7] The document can be viewed online in its translated and non-translated form.[8] From

this document, it can be surmised that the Egyptians were able to discriminate between wounds that were infected and those that were not; they had an understanding of suppurative discharge and promoted the benefits of wound drainage.[5,6,9] When they did cover wounds, they would put honey and grease underneath the lint bandages. Myrrh, byt, and ftt (grease, honey, and lint) formed the standard wound salve of the Smith papyrus and are mentioned over 500 times in this document. Honey was presumably used for its antiseptic properties, and grease was most likely used to help prevent the lint fiber bandages from sticking to the wound.[5,7]

The next major advancement in the management of healing wounds came with the ancient Greeks. The first ligation of blood vessels and use of ligatures during surgery was during this period.[6,7] Hippocrates (c.460-370 B.C.) was one of the most influential men of this period. He changed the fundamentals of wound care by emphasizing that wounds should only be irrigated with water or wine, injured areas should be kept immobilized if possible, and that an injured area should be compared to the comparable uninjured region. Hippocrates was also responsible for the first definitions and descriptions of healing by first and second intention.[6]

Aurelius Cornelius Celsus (c.?7B.C.–37 A.D.) authored the most extensive ancient collection of medical knowledge after Hippocrates, "*De re Medica*".[5,6,10] His textbook consisted of eight chapters, the fourth of which was the first to describe the four cardinal signs of inflammation, *rubor, tumor, calor*, and *dolor*: redness, swelling, heat, and pain. (It would be yet a millennium before the fifth and last cardinal sign of inflammation, *functio laesa*: disturbed function, is described by Robert Virchow in his book *"Cellularpathologie"*, 1858)(**Figure 1.2**).[9] In the

fifth chapter of "*De re Medica"*, Celsus describes the importance of hemostasis and discusses primary closure of wounds with suture or thread.[10]

The very prolific (writing over 2.5 million words) Galen of Pergamon (129-212A.D) is arguably one of the most accomplished wound surgeons and medical researchers of antiquity. Galen started his medical career by performing many dissections in Alexandria, before coming to Rome and being appointed to treat injured gladiators.[6,7,11] Galen showed a strong interest in anatomy, and since human dissection was prohibited by Roman law, Galen spent his time dissecting pigs, primates, and a wide range of other species. These dissections allowed him a more complete anatomic (rather than physiologic) understanding of the circulatory system, nervous system, and respiratory system.[12] His dissections and vivisections led him to distinguish not only the difference between arteries and veins, but also to develop four techniques to control bleeding. These techniques were: direct pressure with a finger, twisting the cut end of the vessel with a hook, tying of the cut end or applying astringent preparations.[9] Interestingly, he was vehemently opposed to the use of the tourniquet to attenuate hemorrhage. Another contribution to wound healing by Galen was to keep a continuously moist environment over wounds. He most commonly used a combination of wheat flour and oil.[6,9]

Following Galen, knowledge of medicine, surgery, and wound care declined through the Dark Ages and the Middle Ages, until the Renaissance. Although some rather sensational wound dressings were applied during these eras (e.g., warm ox urine, turpentine, dove's dung, soot and boiling oil or pitch), several basic tenets of wound care developed and have persisted.[9]

It became standard of care to cleanse wounds, to remove foreign debris, and to dress the wounds with a bandage to keep the outside elements out.[7] Repeated bandage changes were performed and a variety of topical drugs were used to promote cleaning, sealing, and scarring of the tissues.[9]

One surgeon in particular, Guy de Chauliac, does stand out in the Middle Ages. In 1363, he wrote one of the most popular textbooks of surgery of the time, "Cyrugia Magna".[13] At this time, the principles set forth by Galen were still very influential; however Chauliac did have some important contributions of his own. According to Chauliac, there are five steps involved in wound care: "the first requires the removal of foreign bodies…the second to reapproximate the separated parts…the third to maintain the reapposed parts in their proper form, and to unite them together as one. The fourth is to conserve and preserve the substance of member. The fifth teaches the correction of complications."[13] The third step, primary union of wounds, was a commendable goal, but not always possible to achieve at this stage in the history of medicine.[13]

One of the most representative figures of the Renaissance was Ambroise Paré (1510-1590), a military surgeon and the official royal surgeon for Henry II, Francis II, Charles IX, and Henry III. He is most famous for substituting egg yolk, rose oil and turpentine for the boiling oil that was used to cauterize wounds, and for re-introducing artery ligation. The belief at the time was that gunshot wounds during battle were contaminated by powder and in need of cauterization. The use of boiling oil not only cauterized the wound but also burned the tissues, causing long-term and marked pain to the patient. During one instance, after battle, Paré did not have enough

hot oil and substituted the egg yolk, rose oil, and turpentine. He noted that the new treatment was less traumatic and painful for the patient, and the wounds healed better.[5,11,14] Paré was also responsible for the invention of prosthetics for both upper and lower body extremities (**Figure 1.3**).[14]

Huge advancements in wound care did not come again until the 19th century, and one of the most important advancements to medicine and wound healing during this era was the introduction of modern antiseptics and antibiotics.[15] During 19th century, medicine moved from purely macroscopic toward the microscopic. It was also filled with famous scientists such as Louis Pasteur and Joseph Lister, both of whom made significant contributions to modern medicine.

Louis Pasteur (1822-1895) developed the concept that bacteria and germs were ever present in the environment and they did not spontaneously generate, but it was Joseph Lister (1827-1912), chairman of surgery and head of the Royal Infirmary of Glasgow, who was able to further utilize this fact to benefit.[7,11,16] Joseph Lister determined that by using a chemical agent, germs and bacteria in the environment could be decreased in number or eliminated, thereby preventing their spread from the environment into wounds.[7,11,17] Lister was able to disseminate his theories on the subject through his publication on the use of carbolic acid in compound fractures.[15,18] Although initially viewed with some scorn, this publication led to the beginning of antisepsis, and the dramatic decrease of infection and mortality rates in wounded and surgically treated patients subsequently validated Lister´s claims.[19] He began to soak his instruments and

bandages in carbolic acid and spray it in operating theaters before performing surgery. This practice lead to a significant decrease in mortality rates, from almost 50% down to 15%.[7,20] The discovery of antisepsis was one of the most important discoveries of the 19th century, arguably even more important than that of anesthesia. Anesthesia had a huge impact during surgery, but it did not immediately impact the patient´s post-operative survival the way the practice of antisepsis did.[21]

Rudolf Virchow (1821-1912) was considered a pioneer of cellular pathology, and the founder of the role of white blood cells in inflammation.[22] Robert Koch (1843-1910), one of the founders of microbiology, did extensive research with bacteria such as *Tuberculosis bacillus* and *Bacillus anthracis*. It was from studies on anthrax that Koch established his famous postulates in 1876, "1. Found in all cases of the disease examined, while absent in healthy organisms; 2. Prepared and maintained in pure culture; 3. Capable of producing the original infection, even after several generations in culture; 4. Retrievable from an inoculated animal and cultured again."[21,23]

Johnson and Johnson was the first company to start mass producing sterile dressing. The founder, Robert Wood Johnson, heard Lister speak at a medical meeting in 1876, and in 1891 the first sterile dressings were on the market.[5,7] These dressings, in the form of cotton gauze, were impregnated with a solution of iodoform. Other ointment or solutions including iodine, metals, and antibiotics, were also used to impregnate the gauze. The gauze was then placed over the wounds and therefore able to impart their antiseptic properties as well as protect the wound from ongoing contamination.[7] It is interesting to note that aside from the chemical additives, the

materials used for bandaging had not changed since Egyptian times. Although wound cleansing was routinely practiced, saline wound irrigation, however, did not appear to be common until the twentieth century, possibly due to the high cost of salt before then. The practices of saline irrigation of the wound and further debridement with saline-soaked, wet-to-dry dressings were developed in the first half of the 20th century. Since then, wet-to-dry dressings have been one of the stalwarts of managing contaminated and dirty open wounds. These primary dressings entail the application of damp wide mesh gauze directly onto the wound that gradually evaporates over several hours, adhering to the wound and simultaneously drawing away exudate and debris by capillary action. The dressing has the benefit of being both cleansing and debriding in nature, gathering unwanted wound products within the interstices of the gauze.[7]

A surgeon by the name of Antoine Depage (1862-1925) can be credited with developing modern debridement. World War I presented new challenges for army surgeons, high-velocity bullets and shrapnel injuries combined with contamination from the battlefield led to highly soiled wounds.[5] During his treatments he not only removed foreign bodies, but he also removed contaminated tissue and necrotic flesh. His insightful rationale was that he believed the damaged tissue to be a perfect breeding ground for the bacteria responsible for gas gangrene.[5]

As we have advanced into the 20th century, our deepening understanding of the mechanisms of wound healing has been our greatest asset. New developments in wound management have been based on helping the body to heal itself, especially in patients with comorbidities. Newly

developed, therapies, pharmacological, biological and mechanical, help to enhance the biologic pathways of tissue repair; including: angiogenesis, cell migration and proliferation, protein synthesis and tissue remodeling. New technologies support these pathways and are based on biophysical stimulation (examples include pulsed electromagnetic fields and laser irradiation) or exploiting a controlled environment (examples include: negative pressure wound therapy, hyperbaric oxygen),[11,24,25] many of these novel therapies and adjuncts remain to be fully validated in the clinical situation.

PHASES OF WOUND HEALING

Continued advances in molecular science have allowed us to more fully understand the depth and breadth of what is happening at the cellular and subcellular level. As this knowledge of cellular interactions grows, so does our understanding of wound healing. Modern knowledge of the phases of wound healing allows a clinician to treat patients effectively, and with the appropriate therapy.

Wound healing is generally described in three overlapping phases: the inflammatory phase, the proliferative phase, and the maturation phase.[26] The inflammatory phase consists of the initiation of inflammation within the wounded tissue and the development of a provisional extracellular matrix. This inflammation is closely followed by removal of impaired or dead tissue and wound contaminants.[27] During the proliferative phase, the wounded tissues start to repair. This restoration includes re-perfusion of the tissues, development of the extracellular matrix, fibroplasia, contraction and epithelialization of the wound.[27] During the maturation

phase, also known as the remodelling phase, collagen is reorganized by selective absorption and cross-linking and tissue strength is partially restored.[27] It has been noted that acute wounds and chronic wounds do not go through the three phases of wound healing with the same timeline. Acute wounds seems to progress through the phases with a predictable timeline. However, chronic wounds obviously take longer and do not progress normally, for a variety of reasons related to the patient, the wound, or the management. Chronic wounds have more difficulty progressing past the inflammatory phase and therefore do not return to normal functionality or integrity.[27]

The inflammatory phase begins immediately at the time and site of injury. This phase normally lasts 2-5 days in mammalian wounds. Once a wound is made, and the tissue disrupted, blood and lymph from the damaged vessels begin to fill the site. Almost immediately, endothelin is produced and vasoconstriction occurs due to the release of other vasoactive mediators such as epinephrine, norepinephrine, and prostaglandins, which initiate contraction of the smooth muscle within the vessel walls.[28] This vasoconstriction helps to minimize the blood loss from the newly damaged vessels; however, it only lasts 5-10 minutes before vasodilation occurs.[26] Blood and lymph are then able to flow into the extravascular space, increasing interstitial pressure. This increased blood flow and compressed lymphatic drainage to the wound bed results in the classic signs of inflammation: heat, redness, and swelling.

At the time of wounding, the coagulation cascade is activated and thrombin formed. Thrombin is a catalyst for conversion of fibrinogen to fibrin. This newly formed fibrin is important for

platelet activation.[29] Once platelets are activated they are able to form a plug by adhering to the exposed subendothelial collagen of the injured vessel wall, and thereby attenuating the bleeding.[27] These activated platelets also release alpha granules, which contain several growth factors including: platelet-derived growth factor (PDGF), transforming growth factor- (TGF-β), epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), and fibroblast growth factor (FGF). These growth factors, present within this newly formed provisional extracellular matrix, are important for recruiting and attracting other important cells required for wound healing.[27]

The inflammation is also characterized by the migration of leukocytes (primarily neturophils and monocytes) from the dilated vessels to the damaged tissue. Tumor necrosis factor-α (TNF-α), PDGF, TGF-β, EGF, interleukin-1 (IL-1), complement and bacterial products mediate neutrophil migration and activation.[26,30] Neutrophils first arrive to the wound within 24-48 hours of wounding. The role of neutrophils is to phagocytize bacteria and breakdown extracellular debris, which removes them from the wound. They also secrete cytokines that prolong the inflammatory phase.[26,31] Neutrophils predominate in the wound in the early stages of inflammation; however, because neutrophils have a short lifespan, macrophages predominate with time. Macrophages start as monocytes in the blood stream and the presence of TGF-β helps them to mature to macrophages once in the tissue.[27] Within 48-96 hours after wounding, the macrophage is the predominant leukocyte in the wound. Macrophages are probably the most influential cells within a wound. They are important for the release of important cytokines (IL-1,

IL-6, IL-8, TNF-α) and growth factors (FGF, EGF, TGF-β, and PDGF) that modulate the wound healing process including, mobilization and regulation of fibroblasts, angiogenesis and epithelialization.[26] Macrophages are also responsible for continued phagocytosis of foreign material, secretion of proteases and removal of bacteria. The extracellular matrix is degraded by matrix metalloproteinases (MMP-1, MMP-2, MMP-3, and MMP-9), which are secreted by macrophages within the wound. This degradation allows other cells to move more freely through the tissues, thereby facilitating the continued healing process.[27,32]

The second stage of wound healing, the proliferative stage, occurs within the first week of injury, normally around days 5-7. This stage is arbitrarily divided into three important steps (although they also overlap significantly): angiogenesis, fibroplasia, and epithelialization. The overall accomplishments of this phase are the formation of granulation tissue, contraction of the wound and re-establishment of epidermal integrity. The length of this phase is dependent on the size and location as well as the presence of any comorbidities of the patient that may impede wound healing.[26,27]

Capillaries begin to grow from existing vasculature into the wound resulting in angiogenesis. There are many factors that are responsible for the initiation of angiogenesis. These include: vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), angiogenin, angiopectin I and TGFβ, among others.[26,27,33,34] These new capillaries grow into the wound bed and may be visible histologically as soon as four days after wounding. This microvascular network within the wound bed provides oxygen and nutrients to the cells of the developing

granulation tissue.[27,35,36] The formation of granulation tissue is the ultimate goal of this phase and is extremely important in wound healing. Granulation tissue consists primarily of capillaries, fibroblasts, and an active extracellular matrix containing collagen, hyaluronic acid, and other substances. It is resistant to infections, plays a major role in wound contraction, and provides a conducive surface for epithelialization.

Fibroplasia is defined as the production of the extracellular matrix and collagen fibers by fibroblasts. The PDGF, TGF-β, and EGF released by platelets and macrophages attract fibroblasts to the wounded tissue. PDGF initiates synthesis of type III collagen, glycosaminoglycans, and fibronectin by the fibroblasts. These are important in the formation of the extracellular matrix and ultimately, granulation tissue. These newly formed collagen peptides are modified to form triple helices, which help to provide added strength to the healing tissues. [37] TGF-β plays a very important role by increasing the synthesis of type I collagen, decreasing production of MMPs and increasing their inhibitors.[38] As granulation tissue matures, some fibroblasts undergo apoptosis, and others are transformed into myofibroblasts in response to TGF-β. These myofibroblasts play a role in contraction of the wound by providing focal adhesions and adequate leverage. They attach to each other, to the extracellular matrix, and to the wound edges, and start to contract. Contraction usually begins about 7 to 9 days after wounding in healthy wounds. [39,40]

Epithelialization will occur from the basement membrane of the epidermis if it is left intact. If the basement membrane is not intact after injury, as in full-thickness epidermal and dermal

wounds, then wound epithelialization occurs from the wound edges. Epithelial cells begin to proliferate at the wound edges in response to the EGF and TGF-α secreted by activated platelets and macrophages. These basal epithelial cells mobilize and appear to ´slide´ out from the edges of the wound, minimizing fluid loss and bacterial penetration in the process. This migration may take weeks and the resultant coverage is initially thin and fragile.[26,27] As the process continues, the cells undergo mitosis, enlargement and flattening. Adnexal structures such as glands and hair follicles are not replaced in this process. Therefore, a hairless epidermis can be the final result in epithelialized wounds covering large surface areas. Epithelialization continues until the basal epithelial cells are signalled to stop by contact inhibition. Migration of cells is then stopped but local cellular differentiation and proliferation continues, and the reformation of a basement membrane will occur at this time.[26,27] If the wound is large, the basement membrane may not be robust, and the final epithelial coverage may always remain thin and fragile, prone to ulceration.

The third and final stage of wound healing, the remodelling and maturation phase, is dedicated to the strengthening of the newly formed collagen. Early in remodelling the extracellular matrix is relatively loosely woven allowing cells to migrate within the wound bed.[41] It is during this time that collagen is progressively remodelled and replaced in this phase. The collagen fibers become thicker and more crosslinked, as well as becoming aligned along the lines of tension, which contributes to an increase in overall wound strength. At the start of the remodelling phase granulation tissue is typically composed of about 30% type III collagen. Type III collagen is replaced by type I collagen, by the end of the phase the healing wound is composed of only 10%

type III collagen.[42] As the matrix becomes stronger, the functions of the fibroblasts begin to change.[43] In the presence of a strong matrix, and through the influence of TGF-β, fibroblasts begin to change into myofibroblasts. These myofibroblasts have contractile ability and continue to pull the wound edges together to facilitate continued epithelialization. Maturation and remodelling of the wound can continue for months depending on the size of the wound. A remaining scar is still 15-20% weaker than surrounding normal tissue. By about three months post-wounding the tissue has achieved its maximum strength, which is about 80% of its original strength.[27,41]

TOPICAL WOUND TREATMENTS

It is important to keep in mind that although all mammalian wounds heal in a mechanistically similar fashion, human wounds are very different from veterinary wounds. Wound healing is a very complex process in both species. Human wound healing is more commonly affected by patient comorbidities (e.g., uremia, obesity, smoking, vascular insufficiency). Common wound factors that affect both human and veterinary wound healing include infection, tension, motion, and lymphedema, among others. Because of the species differences in wound healing, it is important to understand that not all of the research and products developed for human medicine are useful in the veterinary setting. Many companies manufacturing dressings and topical medications for human use try to enter into the veterinary market by using the same advertising and product claims, not realizing that their product may not have the same effect in animal wounds. The majority of the wound healing literature is non-controlled, and much of it is comprised of case series, case reports, and practitioner´s testimonials.

Traditional wound care dressings such as wet-to-dry dressings and dry-to-dry dressings are best used in the acute, traumatic, highly exudative wounds commonly seen in veterinary medicine.[44,45] When wound exudate is markedly reduced, more modern wound care dressings can be considered. Alginate and foam dressings are highly absorptive and can be used in the early repair phase of wound healing. Maceration of wounds can occur if these products are used too early in the management process. Other modern wound care dressings, hydrogels and hydrocolloids, are designed to promote epithelialization. When in contact with the tissue, the gels are able to donate or absorb moisture. At this time, neither clinical experience nor experimental studies in veterinary wounds show an advantage of hydrogels or hydrocolloids over traditional dressings.[45]

The extracellular matrix incorporates an organized collagen scaffold into the wound. The putative role of the extracellular matrix is that it promotes adhesion and migration of fibroblasts and keratinocytes.[46] Various topical medications are available that aim to enhance the environment and improve function of the extracellular matrix and the cells involved with wound healing. Topical silver is most commonly used in the treatment of burns, but it has increased in popularity for use in large veterinary wounds due to its wide antimicrobial activity and absence of toxicity.[47] Tripeptide copper complex is used for its effects on wound neovascularization, epithelialization, collagen deposition and wound contraction.[48] Honey has been used in medicine since the Ancient Egyptians and was found in the tomb of King Tutankhamun and was still edible.[49,50] The wound-healing properties of honey have been continually documented

throughout the 20th century, although with the invention of antibiotics it became less popular.[49,50] Honey has been shown to influence the production of inflammatory cytokines by stimulating human monocytes,[51] although much of the benefit of that substance can be attributed to its antibacterial properties. Hyaluronic acid (HA) is a linear polysaccharide comprising alternating glucuronic acid and N-acetylglycosamine residues, and belongs to a group of substances known as glycosaminoglycans (GAG).[52] Research has implicated HA as a contributor to a number of processes of wound healing. Some of these contributions include cell migration and proliferation, organization of granulation tissue, stimulating angiogenesis, and moderating inflammatory responses.[53,54] Platelet rich plasma (PRP) is defined as plasma that contains a platelet concentration that is above the normal physiologic level that can be found in whole blood.[55] Once it is injected into an affected area it is believed to release growth factors locally for several days; thereby accelerating wound healing in that area.[56] These are just a few of the many wound-healing stimulants that are currently being used and researched, others include, stem cells, growth factors, pulsed electromagnetic fields, low intensity laser, topical oxygen, sugar, maltodextrin, and acemannan from aloe vera.

NEGATIVE PRESSURE WOUND THERAPY

One of the most clinically used topical wound treatments in current human wounds care is Negative Pressure Wound Therapy (NPWT). Negative pressure wound therapy is a treatment modality that has become widely adopted for a broad range of clinical applications since its

invention. NPWT is also known by many other names including ‘vacuum-assisted closure’, ´subatmospheric pressure therapy´ and ‘topical negative pressure therapy’.[57]

The therapy involves placing a porous primary dressing (foam or gauze) over the wound bed, and enclosing the wound with a non-permeable drape. This separates the wound from the outside environment, and allows the negative pressure to act only on that area. The enclosed wound is connected to a programmable pump through a fenestrated tube or disc. Once activated, the pump provides an intermittent or continuous suction through the tubing.

For over a century, clinicians have applied suction (often referred to as “cupping”) to infections and all types of wounds: chronic, post-surgical, and traumatic. These events were first detailed in Bier´s Hyperemic Treatment in 1908.[58] More modern uses of NPWT were described in Russian medical literature in the late 1980s and early 1990s[59-61] and also in Fleischmann´s work.[62,63] In 1989, an article written by Chariker et al. described their clinical experiences with NPWT in 7 patients with cutaneous fistulae.[64] They used moistened gauze dressing over the wound, which was connected to an existing vacuum source, such as hospital wall suction. This model advised a pressure of -80mmHg.

There have been many different models introduced since then, one of which was described by Argenta and Morykwas.[65,66] This group investigated wound healing under NPWT in full thickness wounds in swine, and showed increased blood flow levels with 125 mmHg of

subatmospheric pressure and significantly increased rates of granulation tissue formation with both continuous and intermittent application. They also noted decreased tissue bacterial counts and increased flap survival compared to controls when NPWT was applied.[66] They also described their experience with one of the first commercially available models, VAC® produced by KCI, which uses a foam dressing and advised a pressure of -125mmHg.[65] The major differences between the currently available commercial models are the primary layer dressing (foam or gauze).[65]

NPWT was originally designed for the treatment of chronic wounds. About 2% of the human population will develop a chronic wound in their lifetime, and NPWT has profoundly changed the clinical approach to these wounds.[67] Examples of chronic wounds that are treated with NPWT include: long-term dehisced wounds, vascular and diabetic ulcers, pressure ulcers, and venous stasis ulcers.[68,69] Patients that are not ideal candidates for reconstructive procedures are treated with NPWT until their wounds stabilize or their comorbidities are controlled. Before NPWT is placed, it is important that the wound bed is adequately prepared. Regardless of whether the wound is acute or chronic, it should be thoroughly debrided, removing all necrotic tissue.[67]

Over the past decade, NPWT has become extraordinarily popular in human wound management other than chronic wounds.[67,70-77] This modality is widely employed in a variety of traumatic and surgical wounds in humans, and results have been enthusiastically documented with several

hundred case reports and case series.[57,73,77-79] NPWT is even used to treat high-energy soft tissue wounds and fractures sustained in the battlefield. In these situations allowing wounds to heal by second intention is impractical. Placement of NPWT has been utilized to provide a closed environment over the wound and to help create an optimal wound bed for reconstruction.[74,80,81] It has also been useful in anchoring skin grafts and has been shown to increase graft take.[82,83] General surgeons have often used it on open abdomens, while cardiothoracic surgeons have found it useful for sternotomies and sternal sepsis.[84] In more recent years, NPWT has also been employed to treat acute wounds in veterinary medicine.[85-88] Examples of acute traumatic wounds include: shearing and degloving injuries, open fractures, contaminated wounds, hematomas, penetrating wounds, and other trauma. Nonviable tissue is debrided, foreign bodies removed, and hemostasis obtained before NPWT is applied to these wounds.[67]

MECHANISMS ATTRIBUTED TO NEGATIVE PRESSURE WOUND THERAPY

Many different mechanisms to ameliorate wound healing have been attributed to NPWT, even when scientific validation has been lacking. Purported effects of NPWT include increased fibroplasia through microdeformation, enhanced angiogenesis, reduction of wound bacterial load, decreased interstitial edema, blood flow to the wound, decreased hematoma/seroma formation, and increased expression of various cytokines and growth factors.[66,73,89,90]

Increased Granulation tissue

An animal model by Fabian et al[91] created an ischemic wound using four full-thickness wounds on each ear of 41 male New Zealand white rabbits. On each rabbit, one ear was dressed for use with NPWT and the other ear was used as the control, identically dressed but without NPWT components. The rabbits were then separated into four treatment groups. Group One had NPWT with no suction and no hyperbaric oxygen therapy, Group Two had NPWT dressing with hyperbaric oxygen therapy but no suction, Group Three had NPWT dressing with suction but no hyperbaric oxygen therapy, and Group Four had NPWT dressing with both negative pressure and hyperbaric oxygen therapy. Statistical significance was found in comparison of NPWT dressing to suction and NPWT dressing alone for peak granulation tissue and granulation tissue gap both with and without use of hyperbaric oxygen. Hyperbaric oxygen alone did not significantly affect the rate of healing; however, NPWT dressing with suction was found to significantly affect the rate of healing. Wounds treated with NPWT with suction demonstrated a significantly smaller tissue gap, and a significantly greater mean peak granulation tissue than control wounds. Morykwas et al[89] not only looked at the effects of NPWT on formation of granulation tissue, but they also examined differences caused by varying levels of subatmospheric pressure. In a swine model, they were able to look at multiple different wound treatments on the same animal. Each of four pigs was subjected to -25mmHg, -125mmHg, and -500mmHg. They noted that by day 8, wounds treated with -125mmHg had fully granulated. At this same time point, wounds treated with -25mmHg had only granulated 21.2% and wounds treated with -500mmHg had granulated 5.9%. Therefore, it was concluded that wounds treated with -125mmHg had a significant increase in the rate of granulation tissue formation when compared with other subatmospheric pressures. Another recent study by Malmsjö et al[92] also looked at the effects of varying levels of

subatmospheric pressure on granulation tissue formation. Peripheral wounds in a swine model were treated for 72hours with continuous NPWT (-80mmHg), intermittent NPWT (0 to -80mmHg), or variable NPWT -10 to -80mmHg). It was concluded that both intermittent NPWT and variable NPWT resulted in more granulation tissue than continuous NPWT. However, it was also noted that intermittent NPWT caused more discomfort and pain to the patient; therefore, the use of variable NPWT should be researched further. Jacobs et al[93] used a rodent model to investigate whether NPWT promotes the formation of granulation tissue and healing. Wound closure rates were calculated as a percentage of initial wound size. Statistically significant wound closure rates were found at all times points in the experimental group, and by Day 7 the NPWT treated wounds histologically had well organized collagen fibers and fibroblast proliferation when compared to the control wounds

Reduced Bacterial Load

Early studies implied that NPWT could reduce bacterial wound load.[66] Weed et al[94] provided a clinical retrospective report on the effects of NPWT on bacterial load in 25 patients. Using serial wound cultures they concluded that there was not a consistent effect of NPWT on bacterial load. In fact, bacterial load increased significantly with the use of NPWT and remained in the range of 10^4-10^6 bacteria/gram of tissue. A randomized trial by Moues et al[95] looked at 54 patients in need of open wound management before closure. Wounds were randomized to NPWT or moistened gauze therapy. Biopsies were collected to quantify the bacterial load of the wounds. The total bacterial load was comparable in both therapies; however, there was a difference in bacterial species found in the wounds. There was significantly more *Staphylococcus aureus* and

significantly less nonfermentative gram-negative bacilli in NPWT treated wounds. Since its invention, researchers have hypothesized that NPWT will decrease the bacterial load in wounds. To date, there is not significant and continued scientific evidence to support this claim.

Increased Blood Flow

Wackenfors et al[96] used laser Doppler to measure microvascular blood flow to an inguinal wound of pigs during NPWT. Varying levels of subatmospheric pressure were used (-50 to -200mmHg). They noted that NPWT increased microvascular blood flow a few centimeters from the wound edge. Blood flow was actually decreased in the immediate proximity of NPWT, and an area of hypoperfusion was noted. This area increased with increased negative pressures, and was especially prominent in the subcutaneous tissues when compared to the muscle. They concluded that soft and dense tissues react differently to NPWT, and that a lower subatmospheric pressure during treatment may be more beneficial for soft tissue. (-75mmHg for soft tissue as compared -100mmHg for dense tissue). Wackenfors et al[97] produced another study using a similar model. However, in this model, they focused on wounds of the peristernal thoracic area as opposed to the inguinal area. This model resulted in the same conclusions: NPWT increases microvascular blood flow to the soft tissue and muscle surrounding the wound. A hypoperfused zone was noted in the immediate proximity of NPWT and was larger at greater negative pressures. NPWT induces a change in microvascular blood flow that is dependent on the type of tissue, distance from the wound, and pressure applied. In a randomized, prospective study, Chen et al[98] used a rabbit animal model to look at blood flow and edema of skin wounds. They examined the effects of NPWT versus the control in 32 rabbits. Round full-thickness skin

defects were made on the dorsal portion of each ear. At different time points, a microcirculation microscope and image pattern analyses were used to observe the variation in wound microcirculation through a detective window. They determined that NPWT promoted capillary blood flow velocity, increased capillary caliber, and blood volume. It was also noted that NPWT stimulated angiogenesis and endothelial proliferation, narrowed endothelial spaces, and restored the integrity of the capillary basement membrane. Another animal model by Lindstedt et al[99] looked specifically at the effects of NPWT on the blood flow to the myocardium. Laser Doppler velocimetry was used to analyze the microvascular blood flow before and after application of NPWT of -25mmHg and -50mmHg. It was found that both subatmospheric pressures of NPWT significantly increased the microvascular blood flow in normal, ischemic, and re-perfused myocardium. A follow-up study using higher levels of subatmospheric pressure (-75mmHg and -150mmHg) did not induce microvascular blood flow changes.[100] Another animal model used by Petzina at al[101] examined the effects of NPWT on peristernal soft tissue blood flow after internal mammary artery harvesting. The effect of NPWT was investigated on the left side, where the internal mammary artery had been removed, and the right side, where the internal mammary artery was intact. Blood flow to the left side was decreased when the left internal mammary artery was surgically removed; however, skin blood flow was not affected. NPWT induced an immediate and similar increase in wound edge blood flow on both sides at both -75mmHg and -125mmHg. A prospective, randomized study by Timmers et al[102] looked at the effects of varying subatmospheric pressures on cutaneous blood flow of healthy intact forearm skin using two different foam types (black polyurethane foam and white polyvinyl alcohol foam). Continuous negative pressure was used at a range of -25 to -500mmHg. Non-invasive

laser Doppler probes, incorporated into the dressing, were used to measure blood flow. A significant increase in cutaneous blood flow was noted in both foams up to a subatmospheric pressure of -300mmHg, with a five-fold increase with the polyurethane foam, and a three-fold increase with the polyvinyl alcohol foam.

While other studies have focused on increased circulation to the wound edge, Ichioka et al[103] focused on blood flow in the wound bed. An intravital microsope-video-computer system was used to visualize the preserved vessels in the wound bed. Three varying subatmospheric pressures were used (-125mmHg, -500mmHg, and 0mmHg). It was noted that wound bed circulation was significantly decreased when subatmospheric pressures of -500mmHg were used; whereas wound bed circulation was significantly increased with -125mmgHg. The control group (0mmHg) showed not changes in blood flow during the observation period.

Decreased Hematoma and Seroma Formation

Chintamani et al[104] used a prospective, randomized clinical study to compare the amount and duration of drainage between varying subatmospheric pressures following modified radical mastectomy. Fifty patients were part of the full suction group (700g/m2) and thirty-five patients were in the half-suction group (350g/m2). The two groups were comparable in age, weight, and extent of operation. There was not a significant different between the two treatment groups in regard to seroma formation. However, the half-suction group did have the NPWT removed earlier and they had a significantly shorter hospital stay without any increase in postoperative morbidity.

Increased Cytokines/Growth factors

Kilpadi et al[105] used a swine model to investigate the effect of NPWT on inflammatory cytokine levels. Interleukin (IL)-6, -8, 10, and transforming growth factor-β1 were analyzed using ELISA. Levels were measured at the time of wound creation and hourly for four hours. They noted that there was a significantly earlier and greater peak of IL-10 and maintenance levels of IL-6 compared with control wounds. Labler et al[106] described a prospective, clinical, nonrandomized study on wounds treated with NPWT. They looked at 32 patients with traumatic wounds that required temporary coverage. Sixteen patients were treated with NPWT and the other sixteen with Epigard® (Medisave Medical Products, Germany). At each bandage change wound fluid was collected and IL-6 and -8, vascular endothelial growth factor (VEGF), and fibroblast growth factor-2 were measured by ELISA. Significantly higher levels of Interleukin-8 and VEGF were noted in wound fluid from NPWT treated patients. Additionally, histologic examination of biopsies (using CD31 and von Willebrand factor immunohistochemistry) indicated significantly more neovascularization in NPWT treated patients.

Microdeformation with In-Vitro Modeling

In-vitro research has suggested that only cells that are able to stretch will respond to growth factors, whereas cells that are confined and unable to stretch are more likely to undergo apoptosis rather than proliferate.[107,108] Saxena et al[90] used a computer based in-vitro model to explore the effect of NPWT on microdeformation of cells. In Saxena´s model, the pressure, pore-diameter, and pore fraction volumes were all altered to assess the effects of NPWT on material deformations. This model showed deformations of 5-20% strain with NPWT, which are

consistent with previous results shown to promote cellular proliferation. The authors hypothesize that tissue deformation caused by the application of NPWT causes individual cells to stretch, which thereby promotes cellular proliferation in the microenvironment of the wound.

CLINICAL APPLICATION OF NEGATIVE PRESSURE WOUND THERAPY

NPWT is currently being used for many different clinical applications. These include skin grafts/flaps, acute open wounds, sternal infections, abdominal sepsis and wall wound closure, and over closed incisions.

Grafts/flaps

One of the first articles published on the subject of skin grafting, after 2000, was in 2002 by Scherer et al.[109] This Randomized Controlled Clinical Trial (RCT) examined sixty-one patients with wounds in need of grafting caused by trauma or thermal tissue loss. After split-thickness graft placement, 44% of the patients received standard bolster dressings, and 56% received NPWT. Although there was no significant difference is split-graft take, they did note a trend toward improved with NPWT. Fewer repeat split-grafts were needed in individuals using NPWT, as compared to those with standard bolster dressings. Moisidis et al[82] conducted an RCT looking at 22 patients with wounds requiring grafting. In all cases, split-thickness grafts were used to cover the wounds. Both qualitative and quantitative graft take was analyzed. They found a significant improvement in qualitative graft take when compared to standard bolster dressings; however, the quantitative graft take was not found to be significantly different. Vidrine et al[83] studied 45 radial forearm donor sites that used either NPWT or conventional

splinting to immobilize split-thickness skin grafts. They noted an overall improvement to skin graft take with NPWT. However, the improvement was not statistically significant when compared to skin graft take with standard bolster dressing. A study by Llanos et al[110] looked at sixty patients that had wounds with skin loss that precluded primary closure. Wounds from both the control and experimental groups were dressed with three sheets of polyurethane with a silicone fenestrated tube, translucent adhesive dressing, and flexible gauze. The only difference between the two groups was that the control group was not hooked up to the negative pressure machine. They found that NPWT significantly decreased the loss of split-graft area and shortened the overall hospital stay. Ben-Amotz et al[86] looked at the use of NPWT in distal extremity wounds of 15 dogs. The wounds were closed with a variety of grafts or flaps (saphenous conduit flaps, free skin grafts, and mesh grafts). They found NPWT to be a successful method of securing skin grafts and flaps over a wound bed. The reader is also referred to Chapter 3 of this thesis, where the effects of NPWT on skin graft acceptance in dogs are detailed.

Open Wounds

In 2011, Demaria et al[111] looked at the effects of NPWT over acute open wounds in 10 dogs in a controlled experimental study. They determined that NPWT enhanced and accelerated the formation of granulation tissue. With the use of NPWT, granulation tissue appeared earlier (Day 3 as opposed to Day 7), and was of better quality (smooth) in the NPWT wounds. Exuberant granulation tissue was observed more frequently in control wounds. However, it was also determined that prolonged use of NPWT (use for more than 10 days) inhibited wound

contraction and epithelialization. Overall the authors concluded that NPWT was an effective adjunct to wound healing early in the post-wounding period. Stannard et al[112] conducted an RCT to look at the use NPWT over severe open fractures in humans. The study involved 23 patients with 25 fractures randomized to the control group, and 35 patients randomized to the NPWT group. Both groups underwent initial irrigation and debridement of their wounds. A standard fine mesh gauze dressing was then applied. Repeat irrigation and debridement was repeated every 48-72 hours. The only difference between the control and experimental groups was that the NPWT was applied between irrigation and debridement procedures. Unlike other studies, NPWT did not decrease the time until the wound was ready to be closed. However, they did note a decreased rate of infection and wound dehiscence in patients treated with NPWT. In a study by Bollero et al,[113] NPWT was used as a way of preparing the wound bed for closure. Use of NPWT allowed for fast development of healthy granulation tissue, however the article does not mention how fast. Once healthy granulation tissue was present, the wounds were covered with split-thickness grafts.

Sternal Infections

Postoperative sternal infections, sternal instability, and mediastinitis remain some of the most feared complications in cardiac surgery.[67] The standard of treatment has been the use of myocutaneous flaps to close the defects after serial debridement; however, many cardiac surgeons have begun to use NPWT as their primary treatment for sternal infections.[114]

Gustafsson et al[115] looked at the use of NPWT in 16 patients that had deep sternal wound infections after cardiac surgery. The median duration of NPWT was 9 days, and the median hospital stay was 22 days. They also measured the C-Reactive protein level and noted a steady decline during treatment reaching 45mg/L (Range: 20-173mg/L) at surgical closure. Another study by Fuchs et al[116] also included C-reactive protein level measurements in their results. This retrospective analysis looked at 68 cases of sternal wound infection. Baseline characteristics and blood factors did not differ between the two study groups at diagnosis of sternal infection. It was noted that C-reactive protein levels declined in both treatment groups; with a significantly more rapid decline in the NPWT treatment group. They also noted that the in-hospital stay was shorter, the rewiring of the sternotomy was earlier, and the bacterial load was reduced more quickly in the NPWT treatment group when compared to the control group. A study by Sjögren et al[117] compared the clinical outcome and survival in 101 patients undergoing NPWT or conventional treatment for poststernotomy mediastinitis. A significantly lower 90-day mortality rate, and failure rate was reported with NPWT. The overall survival rate was also significantly better in the NPWT treatment group. A retrospective study by Halvorson et al[118] looked at the rate of infection in pediatric open fractures treated with NPWT. Twenty-eight patients aged 2-17 were included with thirty-seven open fractures of different varieties that were initially treated with NPWT. The overall infection rate recorded was 5%, this included no superficial infections and two cases of deep infection. It is difficult to draw conclusions from this study because no control group was included.

Abdominal Sepsis and Wall Wound Closure

DeFranzo et al[119] retrospectively looked at 100 patients that were treated with NPWT prior to reconstruction of their abdominal wall. The abdominal wounds were caused by a variety of factors including: gastroschisis, abdominal compartment syndrome, trauma, postoperative dehiscence, necrosis, and infection. Overall, it was concluded that NPWT shortened time to reconstruction as well as helped to simplify the method used for reconstruction. An RCT conducted by Bee et al[120] compared NPWT to Polyglactin 910 Mesh as two different options for abdominal coverage after damage control laparotomy or abdominal compartment syndrome. 51 patients were randomized between treatment groups. They noted no difference between delayed primary closure rates, or fistula rates. It was concluded that both methods for abdominal coverage are adequate. A retrospective study by Wild et al[121] compared NPWT with standard open wound packing for open abdominal wounds after surgery. They determined a 40% reduction in mortality in patients treated with NPWT. Subramonia et al[122] prospectively studied the outcomes in patients in which NPWT was used for temporary closure of their open abdominal wounds. Fifty-one patients were included, 10 treated for laparostomy wounds and 41 for abdominal wound dehiscence. Over the course of the study, 18 patients had no complications, 12 patients developed incisional hernias, 9 were lost to follow-up and 12 died. The authors concluded that NPWT is a useful adjunct in the management of the open abdomen. The lack of control group is a major drawback of this study. Another prospective study by Perez et al[123] looked at 37 patients who were temporarily treated with NPWT for severe abdominal sepsis or abdominal compartment syndrome or both. Abdominal closure was achieved in the majority of patients (70%) with no significant relationship to the amount of time they were

treated with NPWT. One interesting end point in this study was the appearance outcome 1 year after closure. They determined that the aesthetic outcome as determined by the Vancouver Scar Scale, was considerably poorer in the NPWT treated group compared with the control group. Further studies are needed to determine whether NPWT really does have a detrimental effect on scar appearance. In human medicine, scar appearance and formation are an important outcome and could deter some clinicians from using NPWT on their patients. More randomized controlled clinical trials need to be performed to quantitatively determine NPWT´s effectiveness at treating open abdominal wounds.

Closed incisions

The overall success of NPWT in the management of open wounds has led clinicians to start researching its effectiveness over closed incisions. The same NPWT mechanism is implemented with a slightly modified dressing, which is cut to fit over the closed incision. This method will be termed incisional negative pressure wound therapy (INPWT). A recent study by Kilpadi et al[124] evaluated closed incision management of INPWT on hematoma/seroma formation. In a swine model, ventral contralateral subcutaneous dead spaces with overlying sutured incision were created. After four days of therapy (-125mmHg for the NPWT group) significantly less hematoma/seroma formation, with no canister fluid collection, was found in experimental sites when compared to the control. This lack of canister fluid collection may be explained by the significant increase in lymph clearance noted in INPWT treated wounds. Stannard et al[125] conducted two prospective, randomized clinical trials evaluating the effects of INPWT over closed incisions. The first involved INPWT as an adjunct for assisting with the drainage of hematomas, and the second looked at INPWT as an adjunct to healing of surgical incisions of

fractures that are at high-risk for wound healing issues. The first study enrolled 44 patients randomized to either INPWT or control treatment groups. Thirty-one patients were randomized into the control group and they noted a mean drainage of 3.1 days (range: 0-11). Thirteen patients were randomized into the INPWT group and they noted a mean drainage of 1.6 days (range: 0-5). There was significantly less drainage in the NPWT group. However, they did not note a significant difference between groups regarding the need for surgical evacuation of the hematoma. Another 44 patients were enrolled in the high-risk fracture study. Twenty-four patients were randomized to the control group and twenty patients were randomized to the INPWT group. Drainage from the surgical incision occurred from a mean of 4.8 days (range: 0-24) in the control group, and for a mean of 1.8 days (range: 0-6) in the INPWT group. Calculations determined that there was a significant difference between the control and INPWT groups regarding drainage. No significant difference was determined regarding decreasing infection rate or wound dehiscence. Although these results are quite promising regarding INPWT, the overall numbers are too small to make any real conclusions about its effect on decreasing dehiscence or infection. Another prospective, randomized clinical trial conducted by Stannard et al[126] in 2012, looked at the effects of INPWT over closed surgical incisions in 249 patients with 263 high-risk fractures. They investigated the prophylactic use of INPWT for prevention of wound dehiscence and infection. They noted that the risk of infection was 1.9 times higher in the control group when compared to INPWT. They also looked at wound dehiscence after discharge. They observed 16.5% of wounds dehisced in the control group and 8.6% in the INPWT group. This difference in rate of wound dehiscence was significantly different. The results of this study are promising, however further larger studies of this kind are indicated. In addition, this study was spread over four different institutions and several surgeons,

which could potentially have had an effect on the results. A retrospective study by Atkins et al[127] looked at 57 post-sternotomy patients treated with INPWT. In these patients, INPWT was placed over clean, closed sternotomy incisions immediately after surgery and maintained for four days postoperatively. Of the 57 patients, the majority were males, over 50% were diabetic, over 75% were obese, and over 50% were obese and diabetic. In this high-risk group of those treated with INPWT, all patients tolerated INPWT to completion, and there were no cases of deep or superficial sternal wound infections. This finding was quite promising considering the rate of post-sternotomy wound infections can be as high as 33% in this high-risk group. Therefore, the study investigators recommend that INPWT be given strong consideration as an adjunct for high-risk post-sternotomy patients. Two retrospective studies were performed by Reddix et al[128,129] looking at INPWT and its effect in postoperative acetabular fracture wound complications. The first study compared patients treated with INPWT before and after it was made part of the postoperative protocol. Three hundred and one patients were treated for an acetabular fracture between August 1996 and April 2005. These patients were split into two groups "pre-INPWT" and "post-INPWT". Of those 301 patients, 66 were included in the pre-INPWT group, and 235 were included in the post-INPWT group. Four (6.06%) deep wound infections and two (3.03%) wound dehisences were recorded in the pre-INPWT group. Three (1.27%) deep wound infections and one (0.426%) wound dehiscence was found in the post-INPWT group. The post-INPWT percentages are significantly different from the pre-INPWT percentages and from the author´s reported pre-INPWT infection rate of 6.15% (p=0.414). The second retrospective study focused on postoperative wound complications in morbidly obese patients. It has been recorded that morbidly obese patients are five times more likely to develop a deep wound infection after acetabular fracture surgery when compared to non-obese patients.[130] Of the 237 records

reviewed, 19 patients were morbidly obese (BMI > 40) and fit all other criteria. In these 19 patients, no wound complications or wound infections in the perioperative period or during final follow-up were recorded. Both retrospectives show promising results, but more prospective, randomized controlled clinical trials need to be performed before final conclusions about INPWT are made.

CONCLUSIONS

There is no doubt that NPWT is one of the most highly reported researched adjuncts to wound healing, and possibly one of the most promising. Its clinical and economic impact in the last twenty years alone is astonishing. NPWT is an innovative and commercially available concept for the management of difficult to treat wounds and chronic wounds of many etiologies. NPWT has most likely reached such widespread popularity due to its ease of use, assumed safety, and assistance in wound management. NPWT requires fewer bandage changes when compared to conventional dressings, as the exudate drains into the canister and not into the bandaging. The decreased bandage changes and in elimination of bandage odor (due to the sealed environment), means more comfort for the patient.[131]

NPWT should not replace basic principles of wound treatment such as wound cleansing, debridement, lavage, and treatment of infection. However, on the basis of this review it appears that NPWT is as least as effective, and for certain mechanisms or clinical applications, more effective than other wound treatments or the standard of care. Most of the literature that is available, however, is based on data that are lacking in good comparative experimental design. Despite the huge penetration of this modality into the human wound care market

and the large number (close to 1000) of articles published in the literature, there is still a lack of randomized controlled clinical trials. Several mechanisms of action have been suggested, but not all of them have been verified. The mechanisms of action with the most evidence are the ability to enhance granulation tissue growth, promote angiogenesis, and increase blood flow. In human clinical wound care, NPWT use is firmly entrenched, and therefore it is somewhat disappointing to see the relatively few controlled clinical trials and small sample sizes.

To this day, NPWT´s influence on bacterial load is controversial. The few clinical studies available lack controls for confounding variables including systemic antibiotics, surgical debridement, and individual patient immune responses. Therefore, there still seems to be insufficient evidence to the claim that NPWT is able to reduce bacterial colonization of wounds.

NPWT has been shown to enhance graft take. The reasons for this have yet to be elucidated; however, the theory is that NPWT results in increased apposition of the graft with the wound bed, decreased shearing forces, and decreased hematoma/seroma formation. The few studies investigating hematoma/seroma formation alone have not been conclusive. Therefore, further controlled clinical trials need to be performed to fully understand why NPWT seems to result in better take of grafts (See Chapter 3 of this thesis).

The use of NPWT in sternal infections allows the surgeon to decide when to close the wound. This gives more flexibility in treatment and more treatment options. NPWT stabilizes the chest wall so that the need for paralytic agents and ventilator assistance is decreased.[132,133] It can be surmised from the literature that hospital stays are shorter, sternums closed more quickly, and

fewer flaps are needed when treated with NPWT. NPWT does indeed appear to enhance healing of sternal wounds.

Although, NPWT was originally developed for the treatment of chronic wounds, it has evolved to be used for types of wounds. The modality is easy to apply, widely applicable, and the technique is simple. Even though further controlled research experiments need to be conducted to fully understand the mechanisms of action, NPWT has proven to be beneficial in the clinical setting and should continue to be used.

APPENDIX A

Tables and Figures Relevant to Chapter 1

FIGURE 1.1: A page of the Edwin Smith Surgical Papyrus. Photograph courtesy of The History of Medicine Division of the National Library of Medicine, National Institute of Health. (For interpretation of the references to color in this and all other figures, the reader is referred to the electronic version of this thesis)

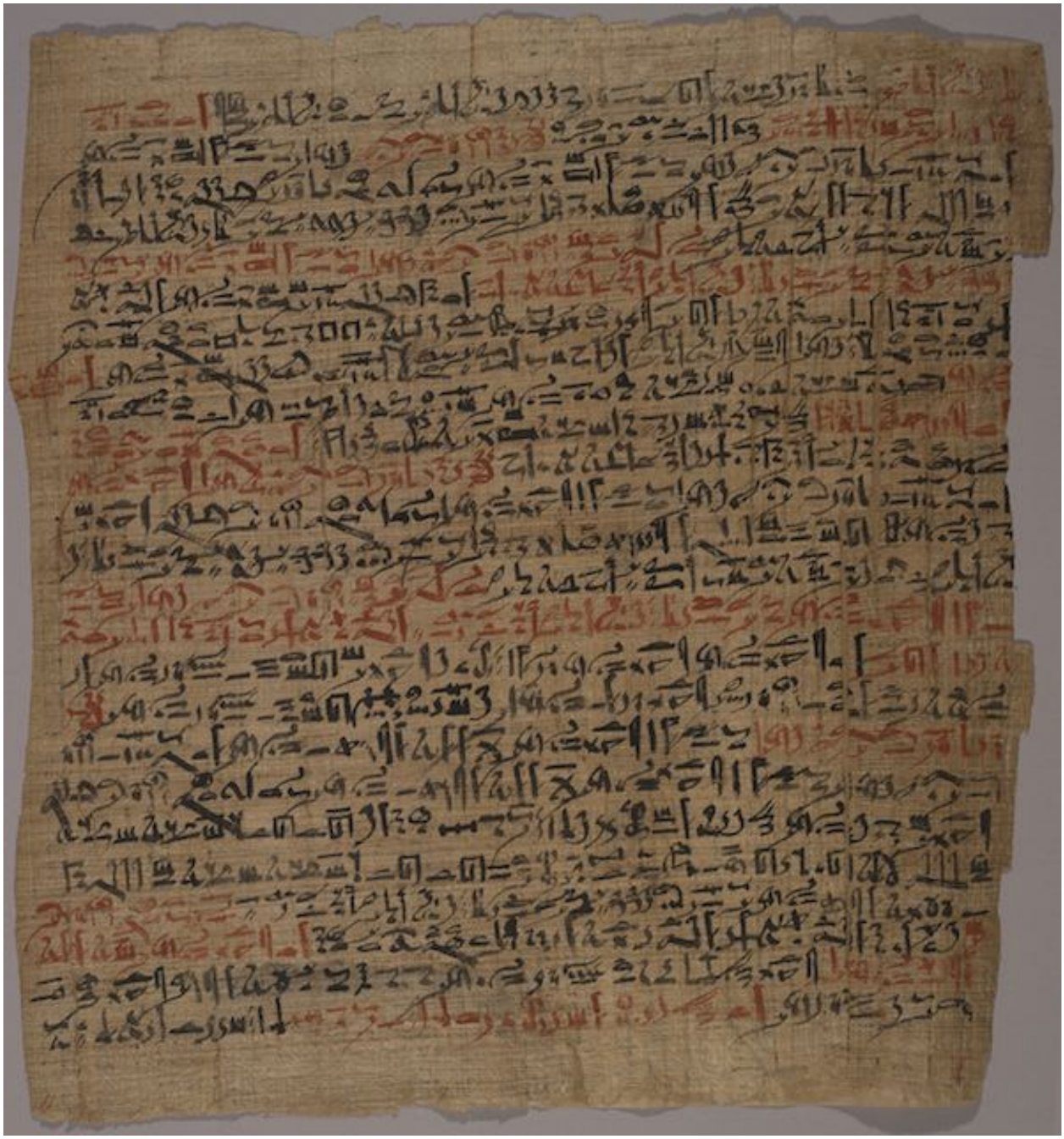


FIGURE 1.2: A cartoon depicting the five cardinal signs of inflammation — heat, redness, swelling, pain and loss of function: This figure was commissioned by D.A.W. and drawn by P. Cull for the Medical Illustration Department at St Bartholomew's Medical College.

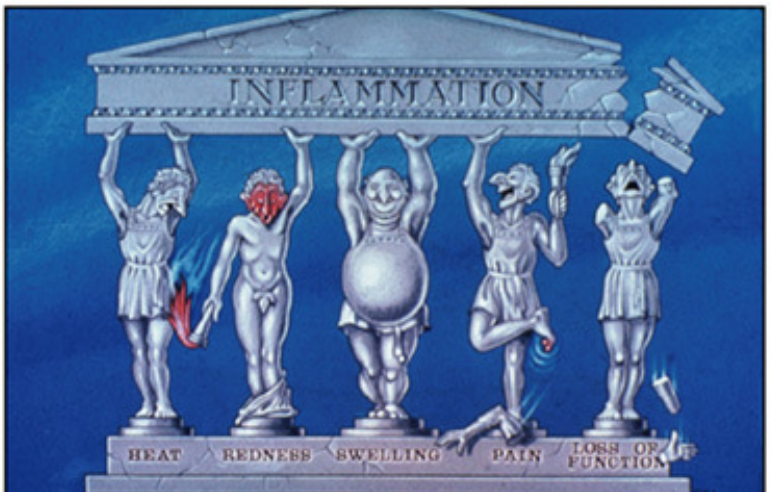
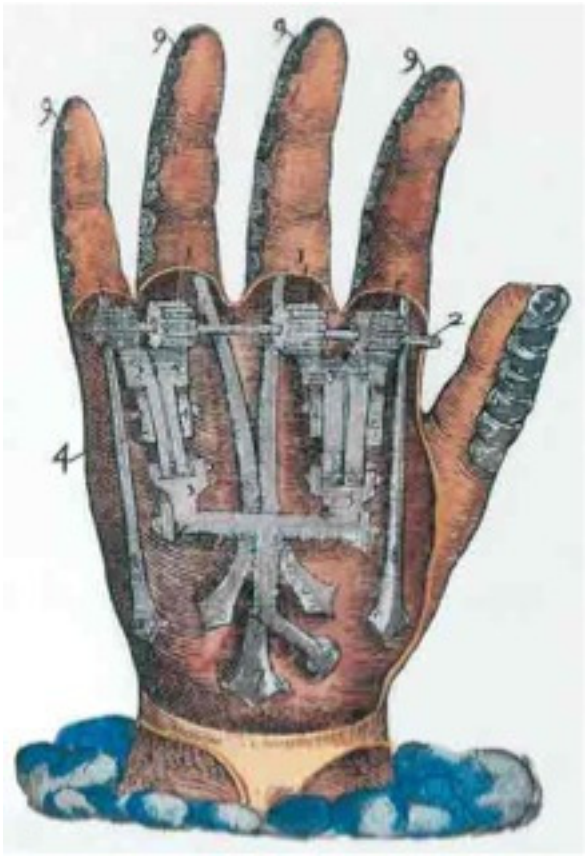


FIGURE 1.3: Diagram of an artificial hand, from Ambroise Paré's *Instrumenta chyrurgiae et icones anathomicae* (Surgical Instruments and Anatomical Illustrations), Paris, 1564



CHAPTER 2: Negative Pressure Wound Therapy: Experience With 50 Veterinary Cases

NEGATIVE PRESSURE WOUND THERAPY: EXPERIENCE WITH 50 VETERINARY CASES

Kathryn A. Pitt, BS, DVM, Bryden J. Stanley, BVMS, MVetSc, DACVS

Department of Small Animal Clinical Sciences, College of Veterinary Medicine, Michigan State University, East Lansing, MI and the Diagnostic Center for Population and Animal Health, College of Veterinary Medicine, Michigan State University, 4125 Beaumont Rd., East Lansing, MI.

Acknowledgements: Chris Phipps, LVT

NEGATIVE PRESSURE WOUND THERAPY: EXPERIENCE WITH 50 VETERINARY CASES

ABSTRACT

Objective: To collate and present data from the first 50 cases using NPWT at this institution, in order to determine if this modality is feasible in referring hospital practice.

Animals: Prospective descriptive study, 45 dogs, 3 cats, 2 horses

Methods: Data collected from the 50 cases enrolled in the study were organized into 6 categories: Patient Data, Wound Data, NPWT Data, Adjunctive Treatments, Complications, and Final Outcome

Results: Fifty cases were recorded, with a total of 58 wounds that utilized NPWT. Seventy percent (70%) had their wounds classified as acute. The remaining 15 cases were classified as chronic. Mean length of treatment with NPWT was 4.40 days. Mean hospitalization time in all cases was 8.20 days. (Range 1-31)

Conclusion: NPWT appears to prepare the wound bed for granulation tissue formation, is well tolerated by patients, allows good mobility, and is a feasible modality for referring hospitals. NPWT is clinically relevant and can be used to optimize the wound bed for second intention healing, delayed primary closure, or secondary closure.

INTRODUCTION

Extensive traumatic cutaneous wounds continue to challenge veterinarians. More complex injuries involving disruption of surrounding soft tissues, bones and joints carry additional management concerns, especially when blood supply is compromised. Patients frequently sustain concurrent life-threatening injuries where repeated daily sedation for wound dressing changes pose additional risks. After patient stabilization, the primary aim of wound management is to rapidly restore functional dermal and epidermal integrity, with minimal morbidity to the patient. The inclusion of a functional dermal layer is particularly important in veterinary patients as the adnexal components contribute to the overall robustness (strength) and appearance of the skin. Final cutaneous restoration is typically obtained via a delayed primary or secondary closure (either direct apposition by some reconstructive effort), although managing the wound until it heals by second intention is also appropriate for smaller wounds. Final wound closure will not be achieved effectively, however, unless the wound is free of infection and debris, and able to produce a healthy bed of granulation tissue.[26,134] Clearly, any treatment that can convert a contaminated or dirty wound into a clean, healthy wound bed in as short a time as possible will greatly benefit both animal and owner.

Negative Pressure Wound Therapy (NPWT), involves the application of a subatmospheric pressure to a wound. It has become widely utilized in human wound care over the last decade, and more recently in veterinary medicine, as an adjunctive therapy before wound closure.[57,86,135-137] NPWT, also termed 'vacuum-assisted closure', 'topical negative pressure therapy', and 'subatmospheric pressure' involves placing a porous primary dressing (open-cell polyurethane ether foam or saline-moistened wide-mesh gauze) into the wound bed, and

enclosing the wound with a non-permeable drape, which adheres to periwound skin. A fenestrated tube or disc is embedded into or on top of the dressing and connected to evacuation tubing (**Figure 2.1**). This converts an open wound into a controlled, closed environment through which an intermittent or continuous sub-atmospheric pressure is applied through a programmable pump. Wound fluid is evacuated through the tubing into a reservoir canister.

NPWT was originally demonstrated in swine studies to increase blood flow to the wound and periwound, remove exudate, and stimulate the formation of granulation tissue.[66,89,138,139] Subsequent investigations have supported some of these findings, such as early granulation tissue formation and increased wound perfusion, but other claims remain to be fully validated.[73,140-142] Nevertheless, NPWT technology has been developed and successfully marketed in the last decade as a mechanical adjunct to wound healing. In the years following its introduction for use on chronic wounds, NPWT use in human wound care has gained widespread popularity and is currently employed in all of the 500 primary care hospitals in the USA.[143] The literature reporting outcomes of NPWT in humans is extensive and currently NPWT is applied to both acute and chronic wounds as well as in surgical applications, such as free skin grafts,[109,144-146] compromised flaps,[147,148] incisional dehiscences,[139,149-151] cytotoxic sloughs,[139] abdominal drainage,[119,152,153] orthopedic trauma,[136,154,155] and burns.[138,156,157] NPWT is also used extensively in the battlefield to address complex soft tissue wounds, and is applied to over 90% of the admitted extremity wounds sustained in the military arena.[80,81,158-

[163] Shorter hospitalization and lower treatment costs have been documented with NPWT.[131,164,165]

Although there are around a dozen different proprietary NPWT systems on the market today (targeting the human wound care industry), the main difference is in the contact layer: this consists of either a foam-based primary dressing or a gauze-based primary dressing. By far the most common system reported in the literature is foam-based, marketed by Kinetic Concepts Incorporated (KCI), San Antonio, TX. KCI holds over 80% of the market share for this modality.[166] Recent studies have shown that both primary dressings have similar mechanisms of action.[167,168]

In contrast to the abundance of human medical reports documenting use of NPWT, the veterinary application of NPWT has been intermittently reported. There are 7 case reports of various species,[85,87,88,169-171] and one retrospective case series of 15 dogs.[86] More recently, two controlled, experimental studies in dogs have been undertaken (See Chapter 3).[111,172] These latter two studies documented the effect of NPWT on the healing of acute open wounds and free cutaneous grafts. When applying NPWT to open wounds over 21 days, it was found to be beneficial in the first week after wounding through the early appearance of a smooth granulation tissue bed. The first appearance of granulation tissue was consistently seen within 3 days under NPWT instead of the 7-9 days of the control wounds. This was supported by the histopathological findings of an earlier onset and subsequent resolution of the acute

inflammatory phase. This early appearance of granulation tissue seen with NPWT, combined with the smoother surface, can shorten the time of open wound management, allowing earlier definitive wound closure through reconstructive techniques such as grafting or flap development. In te study, appeared that after 10 days, NPWT impedes contraction and epithelialization in open wounds in dogs. Benefit was also seen in the application of NPWT for 7 days over free cutaneous grafts (see Chapter 3).[172] Overall, the results of these studies are changing the way we treat acute traumatic open wounds and manage free cutaneous skin grafts in veterinary medicine. The variety of case reports and case series show that the NPWT modality is of interest to those practicing wound care in veterinary medicine. The two comparative veterinary studies document that NPWT is beneficial in the management of open wounds and free skin grafts in dogs.[111,172] What has not been documented in veterinary medicine is the feasibility of learning and using NPWT in a veterinary hospital setting. Information regarding the practical application of the modality, the complications, and pitfalls encountered when incorporating NPWT into the wound care regimen in a clinical setting would be helpful to those considering using this adjunctive modality. These data can be obtained through a large, prospective, observational, clinical study.

There are no published reviews documenting the use of NPWT in a large cohort of clinical patients in veterinary medicine. Based on the rationale that NPWT appear to be beneficial to wound healing. We hypothesized that NPWT would be a feasible, and possibly desirable, adjunct to current wound care protocols in veterinary medicine. The specific aim of this study was to collate and present specific data from the first 50 patients treated with NPWT at Michigan State University´s Veterinary Teaching Hospital.

MATERIALS AND METHODS

Inclusion criteria for this study included all cases with open wounds that underwent NPWT in chronological order of admission until n=50. Since the introduction of NPWT at this institution in July 2006, any animal with an open wound was considered for NPWT application. The final recommendation to owners was made following consultation between the attending clinician and the corresponding author, if different. All wounds were photographed at time of initial presentation and at each dressing change using a high-resolution digital camera (DSC-T200, Sony USA, New York) on macro setting against a carefully positioned metric measurement scale. Data collected for each case were in six categories: patient data, wound data, NPWT data, adjunctive treatments, complications associated with NPWT, and final outcome (**Table 2.1**). The medical records of these first 50 cases were additionally reviewed following data accrual.

Initial wound management protocol

Following any required cardiovascular or respiratory stabilization of the patient all wounds underwent a standardized management protocol before NPWT was applied. Under general anesthesia, the open wound was protected with a sterile water-based lubricant jelly (MediChoice® Lubricating Jelly, Owens & Minor, Mechanicsville, VA) and the periwound skin was liberally clipped for a minimum 10 cm around the wound, and cleansed with chlorhexidine scrub and alcohol (Sun Mark Isopropyl Alcohol®, McKesson, San Francisco, CA; Nolvasan Surgical Scrub®, Fort Dodge Animal Health, Fort Dodge, IA). The open wound was then cleansed with chlorhexidine solution (Nolvasan Solution®, Fort Dodge Animal Health, Fort Dodge, IA) diluted with sterile water to 0.05%. The wound was then gently explored using aseptic technique. The full extent of the wound was identified, hemorrhage adequately

addressed, and any pocketed areas of the wound were carefully broken down so that all wounded areas were communicating. The wound was then surgically debrided using aseptic technique. Copious wound lavage (3L bag minimum) with a sterile, buffered saline solution (lactated ringers) was performed with pulsatile irrigation system (Interpulse®, Stryker, Kalamazoo, MI).

NPWT protocol

During the reporting period, both foam- and gauze-based NPWT commercial systems were used. The foam-based system involved the application of a proprietary polyurethane fether oam, wound sealing kit and suction pad connected to the pump units (V.A.C. ® Granufoam® , T.R.A.C. Pad ®, V.A.C.® Freedom units and canisters, Kinetic Concepts Inc., San Antonio, TX). The two gauze-based systems (EZCare, Smith & Nephew, Largo, FL and Venturi^{TM}, Talley Medical, Hampshire, UK) used saline-moistened, wide-weave gauze into which a fenestrated tube was embedded. The proprietary wound sealing kits (containing primary dressing, tubing, adhesive drapes) were used for all dressing changes.

To prepare the wounded area for NPWT, the periwound skin was dried thoroughly (with a hairdryer on a low setting if needed) and a thin layer of liquid skin adhesive (Mastisol®, Ferndale Laboratories Inc., Ferndale, MI) was applied using a gauze swab (**Figure 2.2**). Using aseptic technique, either saline-moistened gauze was folded into the wound or the polyurethane foam was cut to fit the wound. Attention was paid to ensure that the primary dressing fit just within the margins of the wound to prevent compression of the wound edge (**Figure 2.3**). The evacuation tubing was incorporated into the dressing following each of the manufacturer´s

instructions.[173-176] For gauze dressings, the fenestrated drain was cut to size and tucked within the gauze layers, and the drain secured with a hydrogel or colloid paste to the skin (**Figure 2.4**). For foam dressings, the Trac Pad ® was applied to a fenestration created in the adhesive drape over the foam (**Figure 2.5**). The primary wound dressing was then covered with the manufacturer provided occlusive adhesive drapes. Care was taken to eliminate potential loss of seal by filling all depressions. On wounded areas adjacent to joints, digits and other irregular areas, the use of ostomy pastes, to fill crevices or depression, facilitated application of the adhesive drape (Stomahesive®, ConvaTec USA, Skillman, NJ; Coloplast USA, Minneapolis, MN). The evacuation tubing was then connected to the canister and the pump activated to the appropriate pressure: -80mmHg for gauze based system, -125mmHg for the foam-based system). Successful placement of the drain and adhesive was confirmed by shrinkage, hardening and wrinkling of the dressing once the machine was turned on (**Figure 2.6**). If a leak was suspected, the investigators would listen closely to the dressing for a low, whistling sound to determine its location, and reinforce the seal in necessary. Once an appropriate seal was obtained, the NPWT dressing was covered with a soft, padded bandage, where possible, as this helps to maintain the integrity of the primary dressing. Tubing was arranged and bandaged in such a way that it would allow patient movement (**Figure 2.7**).

RESULTS

Patient Data

Of the 50 cases in the study, 45 were canines, 3 were felines and 2 were equines. Twenty-two different dog breeds were represented, the most common dog type was mixed breed. (**Figure 2.8**). The patient demographic showed 30 male patients (19 neutered) and 20 female patients (18 neutered), with a mean age at admission of 5.6 years (Range: 0.01 – 12.36 years) (**Figure 2.9**). The average weight of the dogs was 31.1kg (Range: 3.1 - 62.3). The average weight of the cats was 6.1 kg (Range: 4.5 - 7.7). One horse (foal) weighed 42.3 kg, and the other horse (standardbred) weighed 297.3kg. The mean recorded BCS of the dogs was 5.2/9 (Range: 1.5 - 8/9). Average hospitalization time was 8.2 days (Range: 1 - 31) (**Figure 2.10**). The majority of patients were treated on NPWT within the hospital setting, however three patients (all dogs) were managed at home with NPWT and came in for dressing bandage changes.

Comorbidities

Comorbidities were widely varied and depended on the individual animal´s health before injury, as well as the cause of the injury. Some of the most common comorbidities, and the comorbidities that can have the most effect on wound healing are: orthopedic injuries (14/50), endocrinopathies (4/50), sepsis (3/50), respiratory disease (4/50) pneumonia, neoplasia (2/50), coagulopathy (3/50), concurrent infection (7/50), renal disease (2/50), failure of passive transfer (1/50), shock (2/50), hepatic disease (1/50), cardiovascular disease (1/50), neurologic disease (2/50), and wound lymphedema (3/50). Some of the less relevant comorbidities include conjunctivitis (3/50), arthritis (2/50), lumbosacral disease (3/50)

Wound Data

The median age of all wounds presented was 5.5 days (Range: 0 - 365). Thirty-five of the 50 cases (70%) had no granulation tissue present; these wounds presented an average of 4.2 days (Range 0 - 21) following wounding. The remaining 15 wounds had granulation tissue present and were considered chronic; presenting at an average of 91.5 days (Range 7 - 365) following injury. Four wounds were >140 days old. Excluding the latter 4 extremely chronic wounds as outliers, the average age of all wounds was 10.4 days (Range: 0 - 98), and average age of the wounds with granulation tissue was 30.2 days (Range: 7 - 98). Of the four wounds that were >140 days old, three were classed as chronic, non-healing wounds by the previous veterinarian before being referred to MSU. The fourth was an abdominal abscess in a horse that had been walled off.

Of the 50 patients in the study there were 58 wounds that used NPWT as part of their management. The etiology of the wounds was varied, with 31 of the 50 cases (62%) having wounds of traumatic origin (vehicular trauma, bite wounds, gunshot, or unknown trauma). The remaining wounds included incisional infections, hygromas/pressure wounds, abscesses, envenomizations and chronic non-healing wounds of unknown origin (**Figure 2.11**).

All wounds extended to full thickness skin loss and were described as lacerations (14%), punctures (18%), dehiscences (10%), physiologic deglovings (4%), anatomic degloving/shears (28%), abscesses (14%), and chronic non-healing open wounds (12%). The wounds were distributed fairly evenly to the trunk, proximal and distal limb, with only 2 wound on the head/neck location (**Figure 2.12**). Fifty-Two (52/58; 89.6%) of the wounds were larger than 5

cm in largest diameter; 29 of them (50%) were greater than 10 cm in largest diameter (**Figure 2.13**).

NPWT Data

Twenty-six of the 50 cases (52%) were treated with foam-based NPWT (at -125 mmHg), and 24/50 cases (48%) received the gauze-based NPWT (at -80 mmHg). Patients waited an average of 2.2 days (Range: 0 – 14) from admission until placement of NPWT, but due to the chronicity of some of the wounds at presentation, the average time from wounding to placement of NPWT was 32 days (Range: 0 – 368). The median length of time on the NPWT modality was 3 days (Range: 1 – 22) (**Figure 2.14**). Manufacturers' recommendations for use of NPWT changed over the course of the study period, thus the earlier cases (n=5) were managed with intermittent suction (5 minutes on, 2 minutes off) whereas more recent cases (n= 45) were managed with the continuous setting. In every case, dressing changes were consistently performed every 2 or 3 days (Mean: 2.3 days; Range: 1 - 3.5 days), for both foam and gauze-based systems. Forty-one animals required heavy sedation or anesthesia for their dressing changes. A consistent sedation protocol was not recorded in 9 cases. Where the protocol was consistently recorded, the most commonly used drugs were acepromazine (50%), hydromorphone (48%), and propofol with isoflurane (40%). Additional drugs used were fentanyl boluses (8%), butorphanol (18%), ketamine and diazepam (24%), and medetomidine (6%). Xylazine was additionally used for dressing changes in the 2 horses. The dosage rates of these drugs as detailed in **Table 2.2.**[177,178]

Definitive closure method in 18 of the 58 (31%) wounds was delayed primary closure (defined as before the presence of granulation tissue); with the same number (18/58; 31%) undergoing

secondary closure (defined as after the appearance of granulation tissue). Definitive closure procedures included direct apposition, skin flap or free cutaneous graft. Twenty-two (38%) of the wounds were allowed to heal by second intention. In these cases, NPWT was discontinued once a smooth, vascular granulation tissue bed was evident which was at an average of 5.4 days. From then on the wound was managed with a variety of traditional semi-occlusive, non-adherent wound dressings either petrolaturm-impregnated knitted cellulose acetate (Adaptic®, Johnson & Johnson, Arlington, TX) or polyester wound contact film polymer with a core of 100% cotton (Telfa ™, Covidien, Mansfield, MA). In the cases that underwent surgical closure, the NPWT machine was removed either immediately before, or the day before, the reconstructive effort. When free skin grafts were applied, however, NPWT was re-applied over a layer of non-adherent dressing (Adaptic®).

Adjunctive Treatments

Adjunctive treatments varied depending mainly on comorbidities and concurrent illnesses and the severity of the injuries to the patient. All patients were on systemic antibiotics at some time during their hospital stay. Common antibiotics used were Clavamox, Cephalexin, and Enrofloxacin (**Table 2.2**). Adjunctive treatments included orthopedic repairs (7/50), blood transfusions (3/50), topical oxygen emulsion (1/50), teeth extractions (2/50), hyaluronic acid (2/50), nasogastric tube placement (1/50), abdominal drains (2/50), and partial rib resection (1/50).

Complications associated with NPWT

Complications associated with NPWT systems were minor, especially as clinicians and staff gained experience in applying and utilizing this modality. The machines themselves did pose some specific complications. These machines were developed for human medicine and therefore have a lot of modes and features that are not necessary in veterinary medicine (e.g., intensity setting, tampering lock). This led to some early concerns understanding the pump alarms. Additionally, although no machine was broken during the study period, they were not designed for robust veterinary use.

Other early challenges included loss of vacuum due to inadequate periwound adhesion, especially in areas such as the digits, where crevices and movement can cause drape detachment and leakage. Meticulous attention to clipping and cleansing the periwound skin, using a spray adhesive and creating a dam across the uneven surfaces (such as interdigital areas) with stoma paste enabled us to overcome this issue. Chewing or kinking of the tubing also caused failure of the device in 3 cases. Appropriate attention to E-collar placement and cage confinement addressed these issues. Once these management and technical issues were resolved, management became much easier. In one case, granulation tissue was noted to adhere slightly to the dressing (immediately before reconstruction) – this dog was 9 years of age, with a large, abscessed elbow hygroma. Additional observations included the intolerance of 2 cats to intermittent therapy - there was sometimes flinching, and occasionally vocalization, when the pump activated. All other cases tolerated both continuous or intermittent NPWT without any other detectable adverse reaction. Three of the 50 cases (6 %) developed mild skin irritation with prolonged NPWT (after 7 - 10 days), although this did not appear to concern the patient. This complication was reduced

by allowing the original adhesive drape to remain on the periwound skin, and just cutting off the dressing immediately over the wound bed, and any other non-adherent portion (**Figure 2.15**).

Final Outcome

The overall outcome for 53/58 wounds (91.4%) was healed. These comprised 23/26 dogs on foam-based and 22/24 dogs on gauze-based NPWT. Nine wounds had minor complications following closure, including seroma (2) superficial flap tip or partial superficial graft necrosis (3), partial incisional dehiscence after primary closure (1), and wound lymphedema (3). All of these complications resolved and the wounds healed. One dog was lost to follow up. Four cases had negative outcomes. Three of these patients had severe concurrent injuries: two patients (one feline, 1 canine) experienced cardiac arrest before final closure could be attempted, and one patient (canine) was euthanized due to progression of concurrent neurologic disease. The fourth patient (canine) had an extensive necrotizing wound of the right thoracic limb, axilla and sternum caused by envenomization. The final reconstructive effort (thoracodorsal axial pattern flap) failed and this patient finally underwent successful forequarter amputation.

DISCUSSION

The results of this observational study support the literature, both human and veterinary, showing that NPWT is a useful mechanical adjunct to mammalian wound healing. Additionally, the experiences documented in this study show that NPWT is feasible in a veterinary hospital setting, which dovetails appropriately with the two controlled experimental studies in dogs.[111,172]

In line with previous comparative studies (See Chapter 3),[111,172] we were impressed with the rapid formation of a smooth granulation tissue bed, and the lack of exuberant granulation tissue formation, even in the horses. It appears that this modality is most useful early in wound healing in preparing a healthy wound bed suitable for a reconstructive (**Figure 2.16)**, rather than a long-term management option. Although the average time to placement of NPWT was just over 2 days (Range 0 – 14), this reflected not so much an intentional delay in application, but rather the time taken for the patient to be transferred to the soft tissue surgery service, especially as most cases had concurrent conditions that required treatment by the emergency service. Currently, the emergency service will now apply NPWT at time if initial wound cleansing and debridement. The average time on NPWT before reconstructive effort was just over 4 days, which is, in the authors' opinion, a shorter time than typical. This particular finding of the study is encouraging and suggests that a randomized, controlled, clinical trial comparing times to reconstruction of wounds treated with and without NPWT is indicated. In this study, NPWT also appeared to accelerate more chronic wound beds into the reparative phase, which optimizes the wound environment for epithelialization and contraction, the main processes involved with second intention healing. One of the interesting findings in this study was the lack of cases where NPWT was applied to the head and neck areas, in view of the fact that we often see wound (e.g., bite wounds) in these areas. In humans, application of NPWT over open wounds or skin grafts in the nuchal area not only improves healing, but enables increased patient mobility (neck movement is socially and functionally very important in humans).[146,179] In this study, we found insurmountable challenges in our attempts to obtain a seal and to maintain integrity of the dressings around the head and neck. For using NPWT in this area, new approaches should be considered, especially as we gain further experience in using the devices. Such approaches may

include more extensive clipping of the face, head, and neck, refinement of additional protective padding with loop sutures over the NPWT dressings, using central line access from the pelvic limbs rather than neck area, and more meticulous attention paid to 'building up' the initial application of the dressing.

One of the biggest advantages in using NPWT, especially in the early, highly-exudative stage of wound management, is the avoidance of daily anesthesia to change the traditional wet-to-dry dressings, especially in larger wounds. Although a limitation to this study is the lack of data on wounds that presented to the VTH and did not receive NPWT, it appears that this modality was utilized much more frequently in larger wounds. Almost 90% of the wounds in this study were larger than 5 cm in diameter, and half of them were greater than 10 cm in diameter. Smaller wounds do not tend to pose the same challenges to the veterinarian and are probably less likely to require the intense wound management that NWPT provides. It is critical that the patient is relatively immobile and in minimal discomfort during dressing changes. As can be seen from the types of drugs used in this study, NPWT application and dressing changes required heavy sedation or anesthesia, which can mean up to a 6-12 hour recovery-period. The 3 days between NPWT dressing changes, allows the patient to recover from sedation or anesthesia adequately to eat, drink, eliminate, ambulate, and interact with visiting owners. In comparison, the standard wet-to-dry dressings, which also require heavy sedation/anesthesia, need to be re-dressed every 12 – 24 hours. Animals undergoing such pharmacologic insult every day tend to not eat well (as they are being fasted or are still sedated) nor do they always ambulate effectively. Both nutritional intake (providing calories and glucose) and ambulation (promoting lymphatic drainage) are important for optimizing wound healing. An additional advantage that was noted

with the NPWT system was that strike-through is completely eliminated, as all exudate and wound fluids are collected into the canister.

The majority of the animals in this prospective, observational study tolerated the NPWT well. The negative outcomes seen in 4 cases (2 died, 1 euthanized, 1 converted to amputation) were considered to be unrelated to the use of NPWT, but due to concurrent conditions or technical failure of reconstructive efforts. Protocol for vacuum application mode for NPWT changed as the manufacturer recommendations changed over the years. In 2007 the industry recommendation for open wound management changed from intermittent (5 minutes on; 2 minutes off) to continuous mode. This was for two main reasons; 1), human patients disliking the re-instigation of negative pressure every 7 minutes, and 2), the increased risk of losing the integrity of the sealed wound environment when the negative pressure was off (especially in more complicated dressings, such as in the perineal or face and neck regions). In this study, therefore, some of the earlier cases were managed with intermittent suction whereas the more recent cases were managed with continuous suction. Due to the intolerance to intermittent therapy in cats, we now use continuous mode in this species. We have, however, returned to intermittent mode in open wounds in dogs and other species, based on our clinical perception as well as recent literature that even earlier granulation tissue develops with this mode.[92] A comparison of continuous, intermittent and variable-pressure wave therapies in several species in veterinary medicine is indicated. In this study, 52% of the wounds were treated with foam-based NPWT, and 48% with gauze based NPWT. Due to the high overall successful outcome (91.4% healed), it was not possible to compare the two primary dressing types without greater numbers.

Maintenance of the therapy was quite simple once initial technical hurdles were overcome. The steepest learning curve was in the securing of a good periwound seal in areas where the skin was uneven, e.g., between the digits, over the hock, the perineum. Techniques such as using various stoma pastes, and adding a liquid skin adhesive to ensure adequate periwound adhesion were learned during this study. Interestingly, placing the system over areas of mobility (e.g., axilla, inguinal area, over joints) did not appear to cause a loss of dressing integrity, possibly because of the disruptive shear forces were neutralized by the "splinting" of the area. When possible, the NPWT dressings were covered with a soft, padded bandage for protection. As animal started to heal and became more mobile, they would sometimes start to circle before they lay down. This behavior should be noted, and any twisting of the tubing can be corrected at this time. This modality was suited for use within a hospital setting, with regular checks by attending physicians and (more commonly) technical support staff.

Although human patients are frequently managed in the community with NPWT, in this study there were only three occasions when animals were sent home with the NPWT units in place. These owners were extremely competent in managing their pets' conditions (one was a veterinary technician, one was a human nurse with experience in NPWT, and one was a very competent owner). Our reluctance to send the dogs home with the NPWT units attached was multifold - our concern over the inability of the owner to cope with the various controls, concern over damage to the unit, and a fear of losing the unit. It would be possible, and would further reduce costs to owner, if we could train ourselves and our owners to increase home management of NPWT, where the animals only come in for dressing changes or if a complication arises.

The models we used for this study were designed for human medical use and therefore were moderately large with many functions and settings that are redundant for veterinary medical applications. We suggest that with modern technological advances (allowing lighter, long-life batteries and stronger pump mechanisms) a smaller, lightweight model could be designed to benefit the veterinary market. Testing of such a prototype model in the veterinary market may help refine design elements for eventual release into the human wound care market. A smaller model would need to be quite robust to withstand rough handling by the patient (human or veterinary). A smaller device would then be able to be carried in a halter or harness, and would reduce the complications regarding excess tubing. A new model could also benefit from a simpler programming design. It is reported that the most effective pressures are range between -80mmHg and -125mmHg.[65,67] Although further investigations into optimal pressures for different mammalian species are indicated, we suggest that the veterinary models could be simplified to have only two negative pressure settings (-80mmHg and -125mmHg); with the option of selecting a continuous mode and a variable pressure mode. The variable pressure mode would not be the same as the current intermittent mode, but would cycle between the -125mmHg and -80mmHg. These changes would make NPWT a much more operator-friendly therapy.

At time of submission, the only company marketing NPWT in the veterinary market is KCI Animal Health (a division of KCI, San Antonio, TX). The cost of a KCI Animal Health Freedom V.A.C.® Therapy Unit is $9,950, the canisters are $57 each, and the V.A.C. ® Dressings (includes tubing, T.R.A.C.™ suction pad and adhesive drapes) cost between $60 and $100, based on size. The costs of initial machine purchase and dressing materials need to be offset against the cost of decreased frequency of dressing changes, shortened time to

reconstruction and shorter hospitalization times, as well as any usage charge to owner. At this VTH, owners are charged $100 for application of NPWT, and $52 per day on NPWT. Dressing kit and canister costs are charged to the owner. Cost comparison studies in human wound care have shown decreased overall cost of care when NPWT is used.[114,165,180-182]

Overall, our clinical experience with NPWT over these few years is very positive, concurring with Ben-Amotz and Lanz´s paper of 15 clinical patients,[5] and the dozen case reports. These clinical impressions, however, should be validated with controlled studies comparing NPWT with standard-of-care dressings, before widespread recommendations are made.

APPENDIX B

Tables and Figures Relevant to Chapter 2

TABLE 2.1: Data collected from each of the 50 cases enrolled in this study were organized into six major categories.

Categories	*Data collected*
Patient Data	Medical record, name, owner
	Species, Breed
	Gender status
	Age
	Weight, Body Condition Score*
	Date of admission; date of discharge
	Comorbidities
Wound Data	Age of wound (days)
	Granulation tissue present or absent
	Etiology
	Wound description
	Location ID 1=trunk, 2=head/neck, 3=proximal limb, 4=distal limb
	Location description
	Size ID at widest point: 1<5cm, 2=5-10cm, 3>10cm
	Size description
NPWT Data	Primary dressing type – foam or gauze; pressure setting
	Days from admission to NPWT application
	Days from wounding to NPWT application
	Total days on NPWT
	Number of dressing changes
	Intermittent or continuous setting
	Days between dressing changes
	Sedation protocol for dressing changes
	Definitive closure type
	Definitive closure day
	Days from end of NPWT to reconstruction
Adjunctive Treatments	
Complications associated with NPWT	
Final outcome	

* The Ohio State University: Body Condition Scoring Chart. (http://vet.osu.edu/vmc/body-condition-scoring-chart).

TABLE 2.2: List of common drug dosages used at MSU VTH.

Drug	Concentration	Route	Dosage (Canine)	Dosage (Feline)	Dosage (Equine)
Acepromazine	2 mg/ml - small animal 10mg/ml – large animal	SQ, IM, IV	0.02-.05mg/kg	0.02-0.1mg/kg	0.01-0.05mg/kg
Hydromorphone	2 mg/ml	SQ, IM, IV	0.07-0.2mg/kg	0.07-0.2mg/kg	N/A
Propofol	10 mg/ml	IV (slowly)	2.0-7mg/kg	2.0-7mg/kg	N/A
Isoflurane	99.9%/ml	ET tube/mask	5% induction: 1.5-2.5% maintenance	5% induction: 1.5-2.5% maintenance	N/A
Fentanyl	50 ug/ml – small animal injectable 5-15mg – large animal patch	IV Transdermal	2.2ug/kg	2.2ug/kg	 2x100 mcg/hr
Ketamine/ Diazepam	50:50 mix by volume	IV	1ml/9kg	1ml/9kg	NA
Medetomidine	1mg/ml	IM	5-10mcg/kg	0.001-0.01mg/kg	NA
Xylazine	20 mg/ml – small animal 100mg/ml – large animal	SQ, IM, IV	0.1-0.6mg/kg	0.1-0.6mg/kg	1.1mg/kg
Clavamox	62.5mg; 125mg; 250mg; 375mg tablets; 62,5mg/ml oral solution	PO	13.75mg/kg Q12	62.5mg/cat Q12	NA
Cephalexin		PO	22-33mg/kg Q6-8	22-30mg/kg Q6-8	22-33mg/kg Q4
Enrofloxacin	22.7mg/ml – small animal 100mg/ml – large animal	IV	5-20mg/kg per day	5-20mg/kg per day	5-7.5 mg/kg

FIGURE 2.1: Application of negative pressure wound therapy, showing open cell foam as contact layer through which subatmospheric pressure is applied. Sealing the area with adhesive drapes converts an open wound into a controlled, closed environment through which an intermittent or continuous sub-atmospheric pressure is applied. As the negative pressure is applied, the dressing takes on a hard and wrinkled appearance.

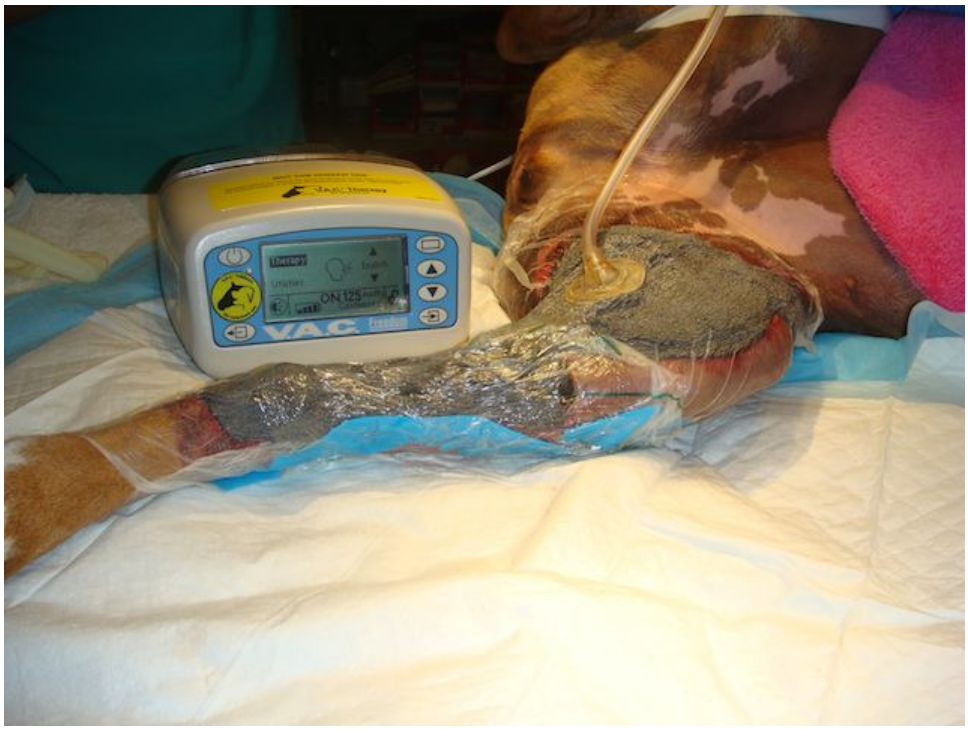
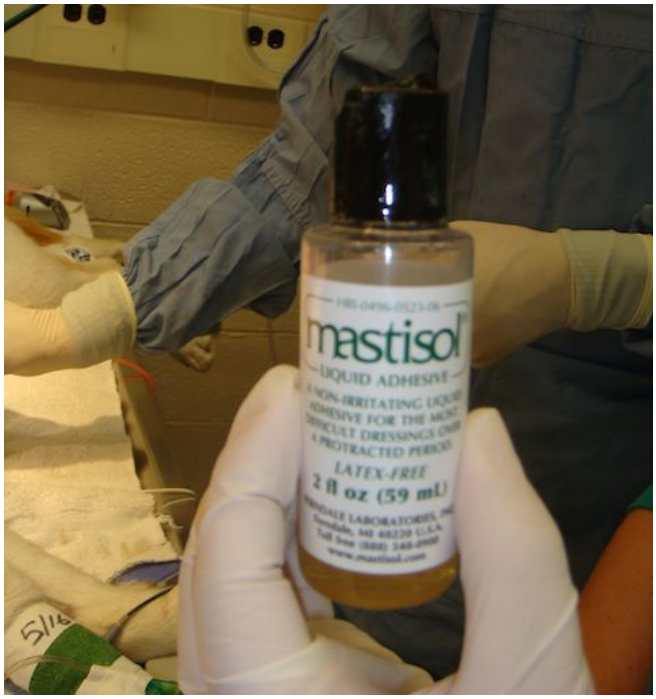


FIGURE 2.2: A thin layer of liquid skin adhesive is applied to the periwound skin. This allows the NPWT dressings to effectively create a seal over the wound.

A)



B)

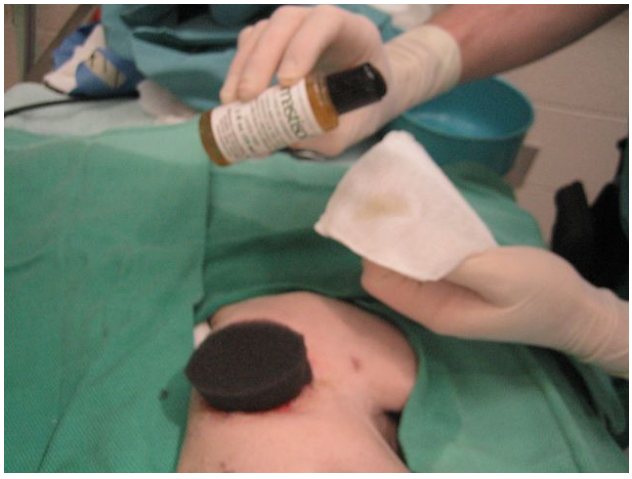
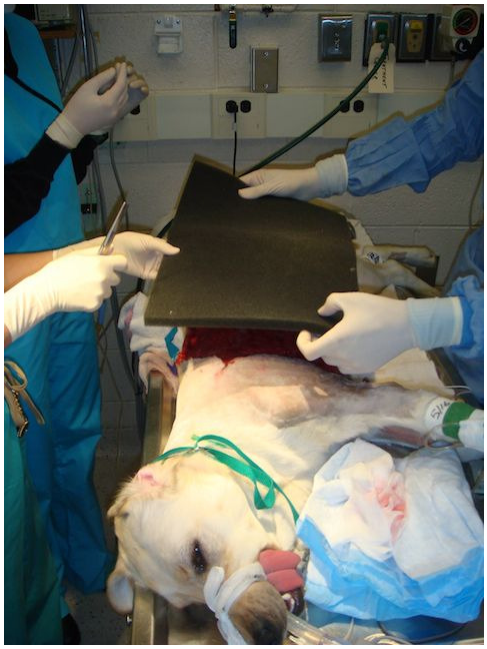


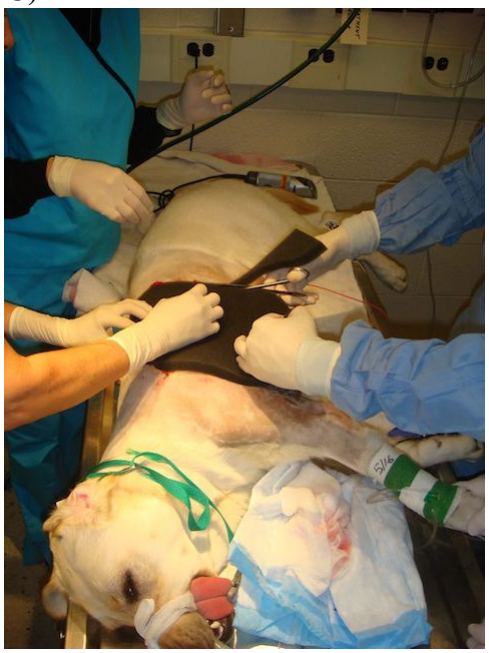
FIGURE 2.3: Primary dressings, in this case open cell foam, are used as the contact layer to cover the wound (A, B). The foam is cut (C) until it fits just within the margins of the wound edge to prevent compression (D).

A)

B)



C)



D)

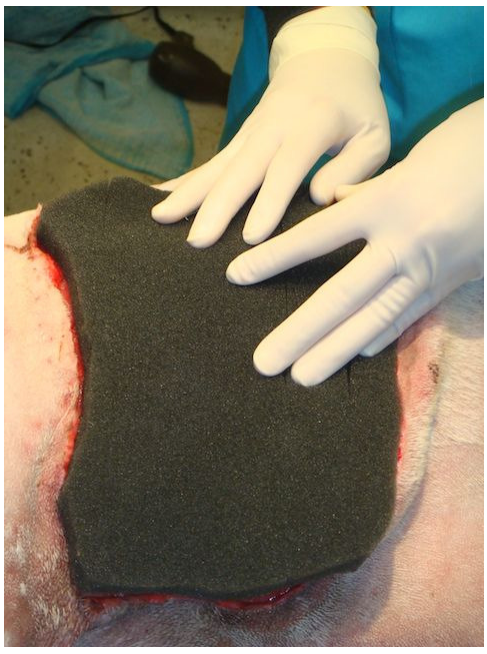
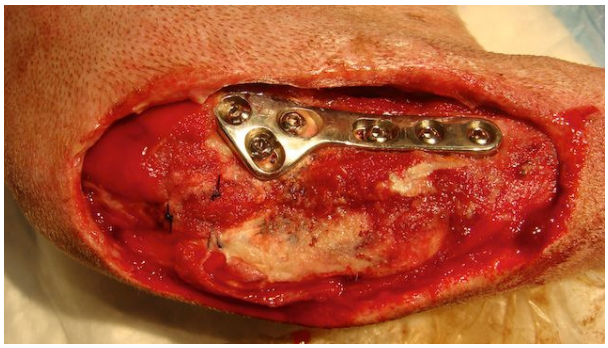
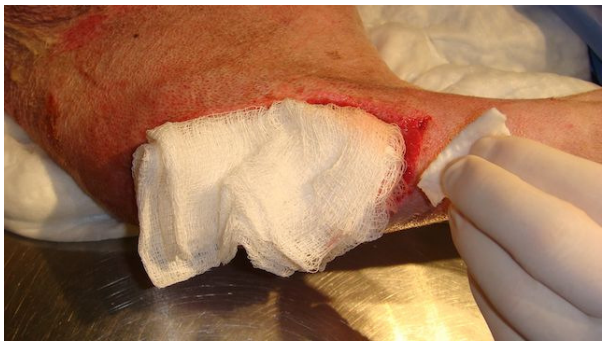


FIGURE 2.4: Dehiscence of an 8-day-old orthopedic surgery site with exposed bone plate (A) is packed with wide-mesh gauze dressings (B). The fenestrated drain is cut to size (C) and tucked within the gauze layers, and the drain secured with a hydrogel or colloid paste to the skin (D).

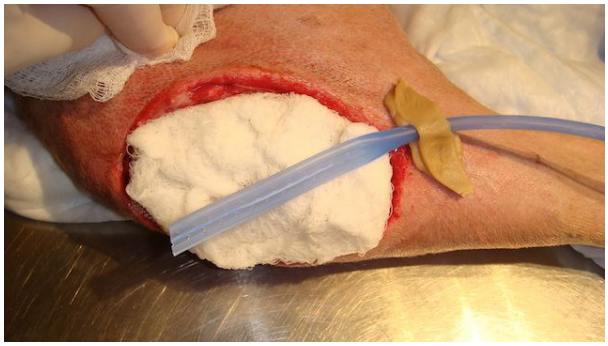
A)



B)



C)



D)

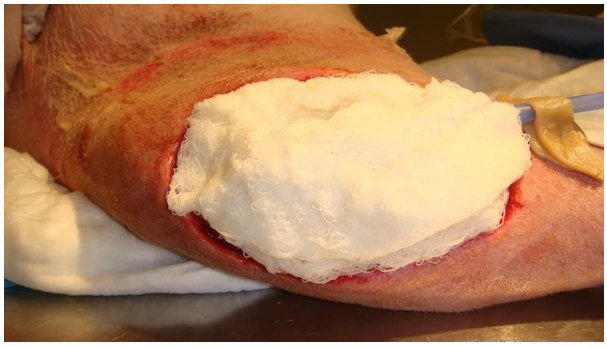
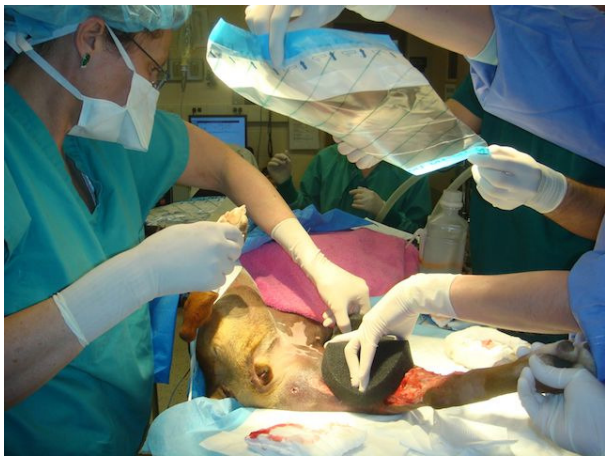
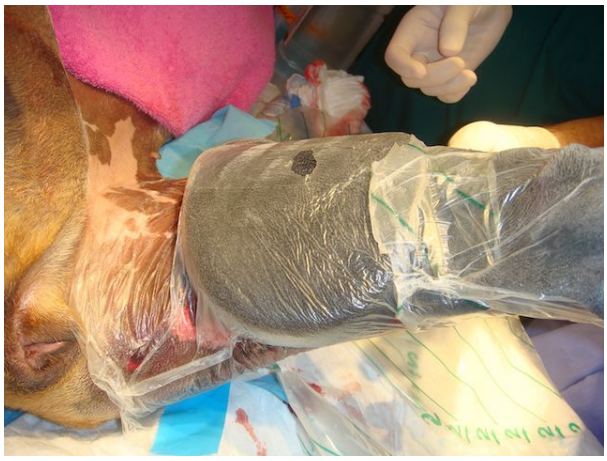


FIGURE 2.5: After being cut to fit the wound edge, an adhesive drape is placed (A). The Trac Pad ® is applied to a fenestration created in the adhesive drape over the foam (B, C).

A)



B)



C)

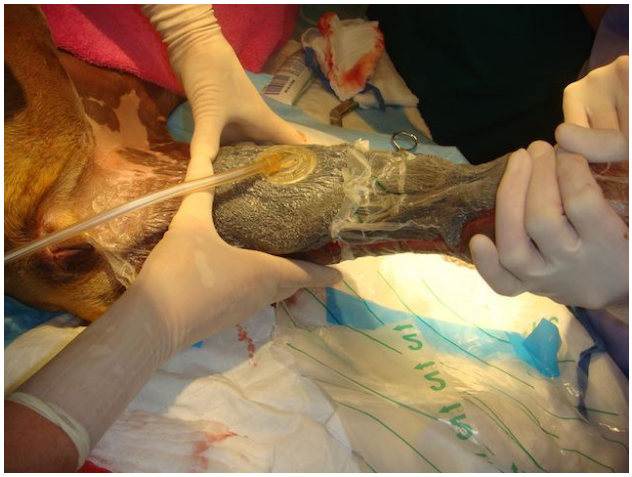
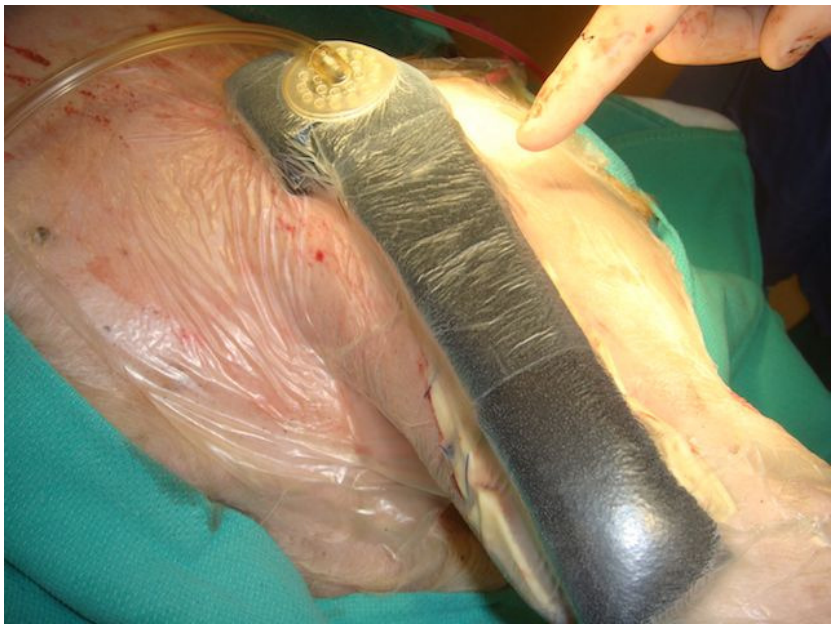


FIGURE 2.6: After the primary layer, drain and adhesive have been placed (A), confirmation of a seal occurs by shrinkage, hardening and wrinkling of the dressing once the machine is turned on (B).

A)



B)

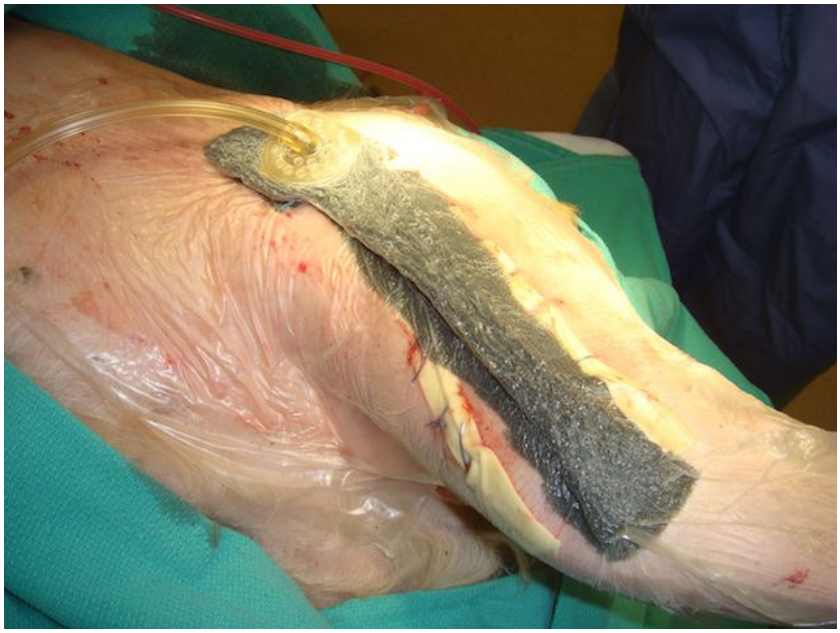


FIGURE 2.7: Machines and tubing are placed in a manner that allows patients to move about while in their kennel.

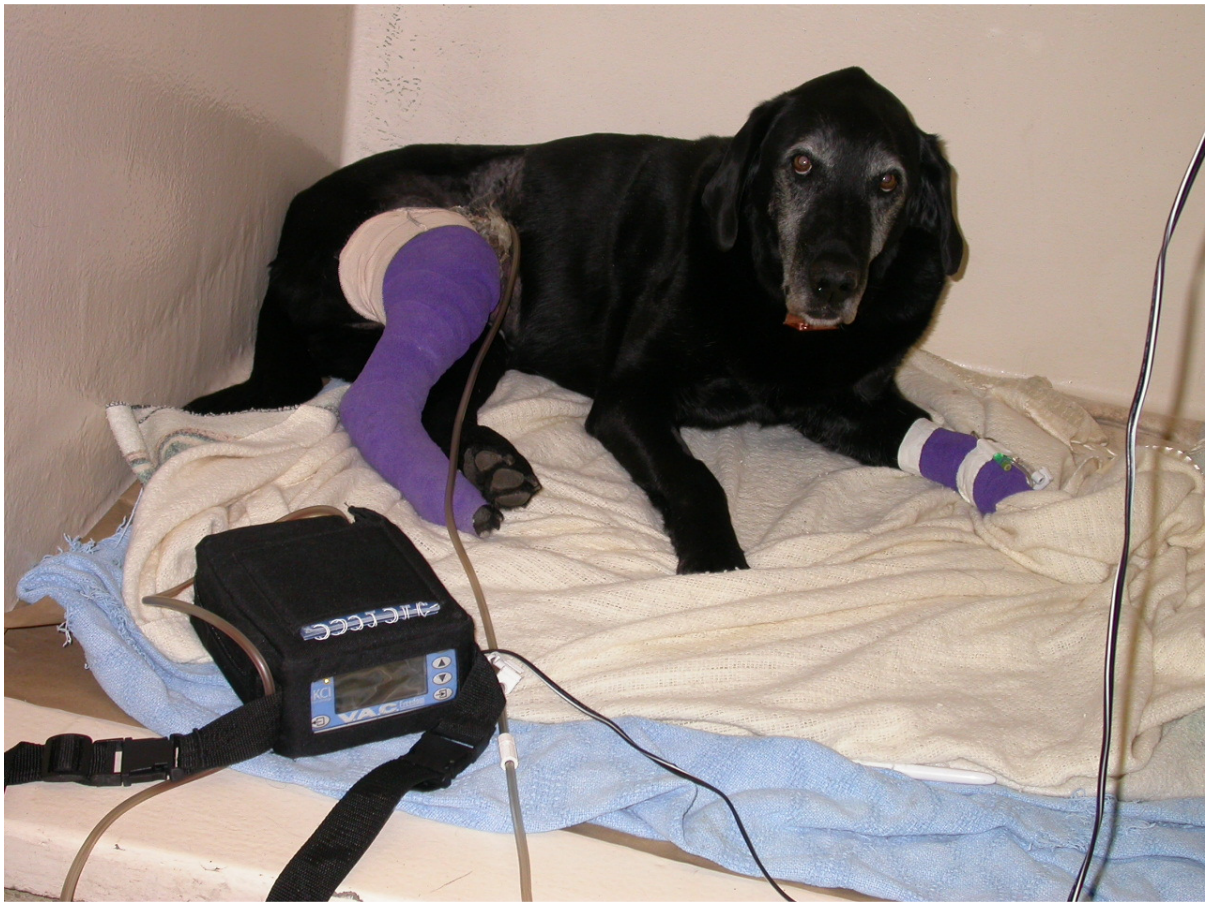


FIGURE 2.8: Species and breed distribution of the 50 reviewed cases undergoing NPWT.

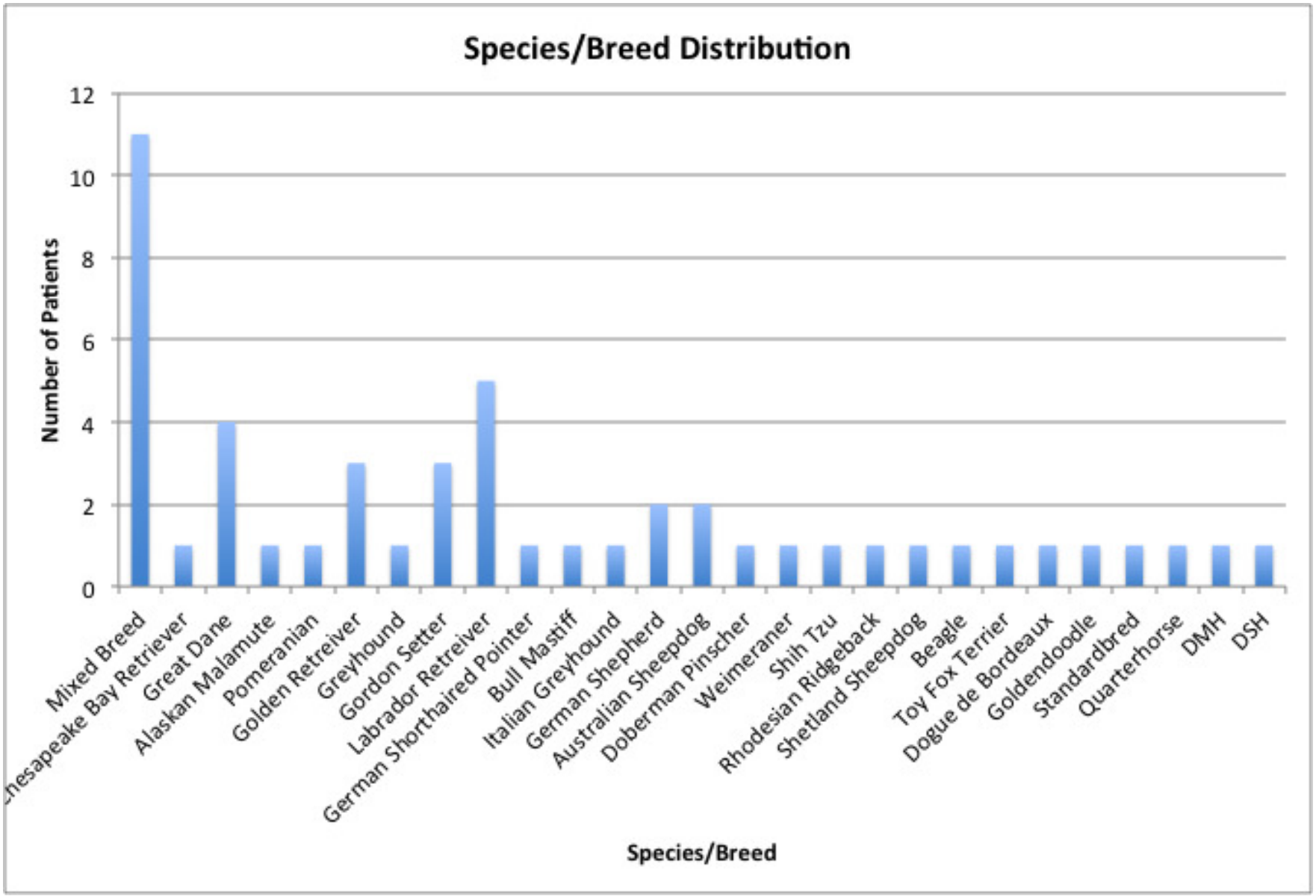


FIGURE 2.9: Age distribution of patients undergoing NPWT. The patient demographic showed 30 male patients (19 neutered) and 20 female patients (18 neutered).

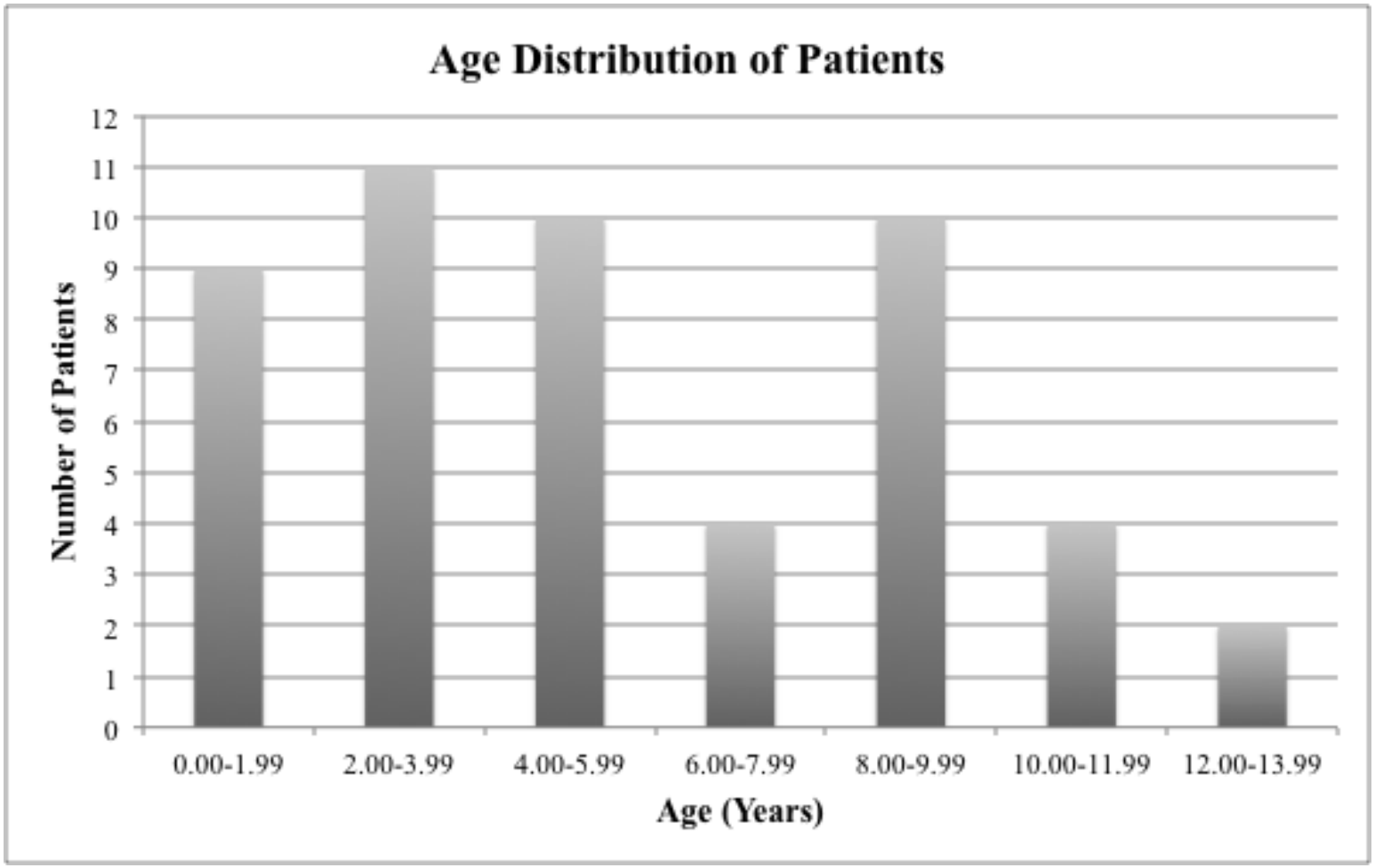


FIGURE 2.10: Patient hospitalization time for the 50 cases undergoing NPWT.

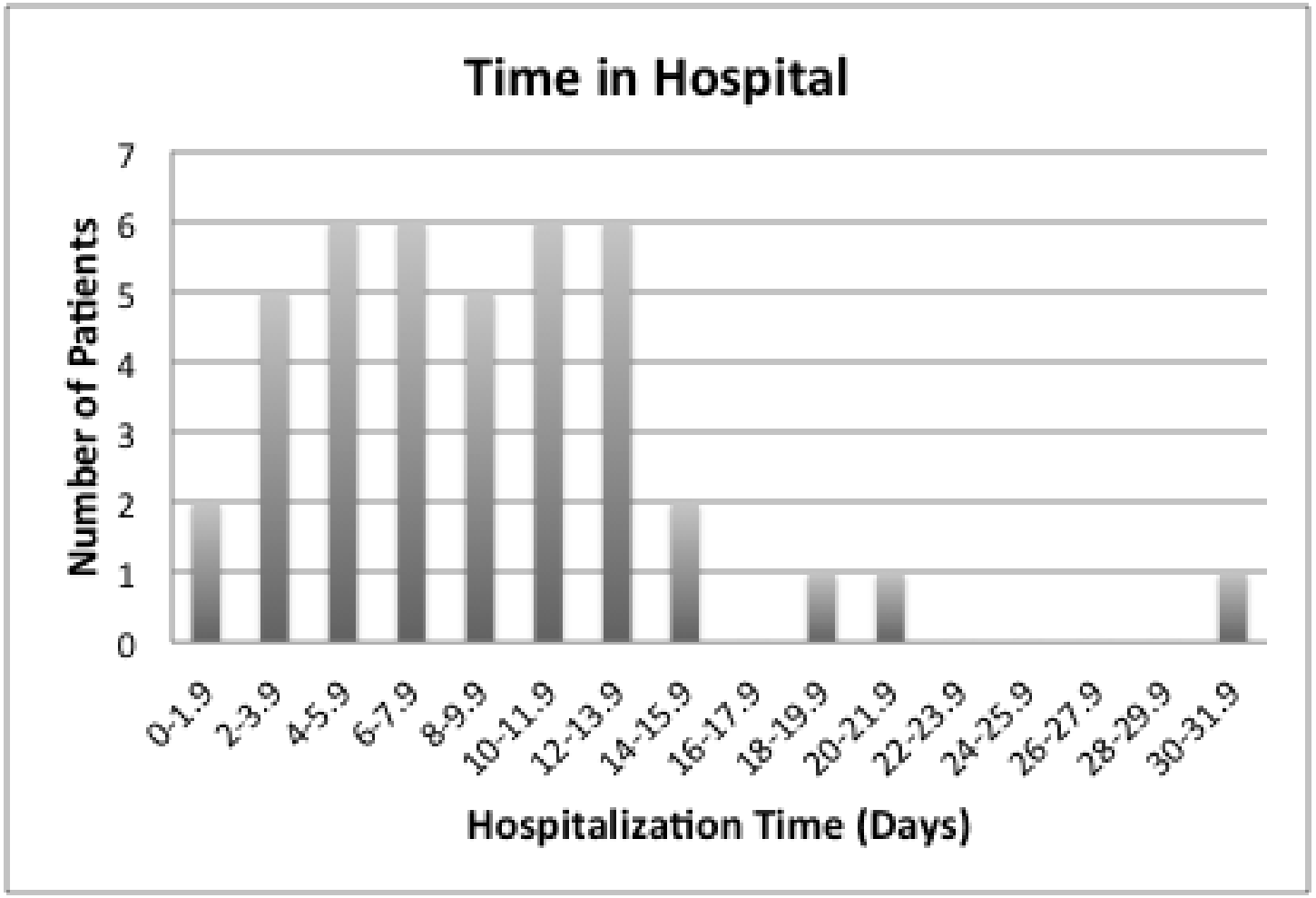


FIGURE 2.11: Pie graph showing the etiology of the 58 wounds from the 50 cases undergoing NPWT.

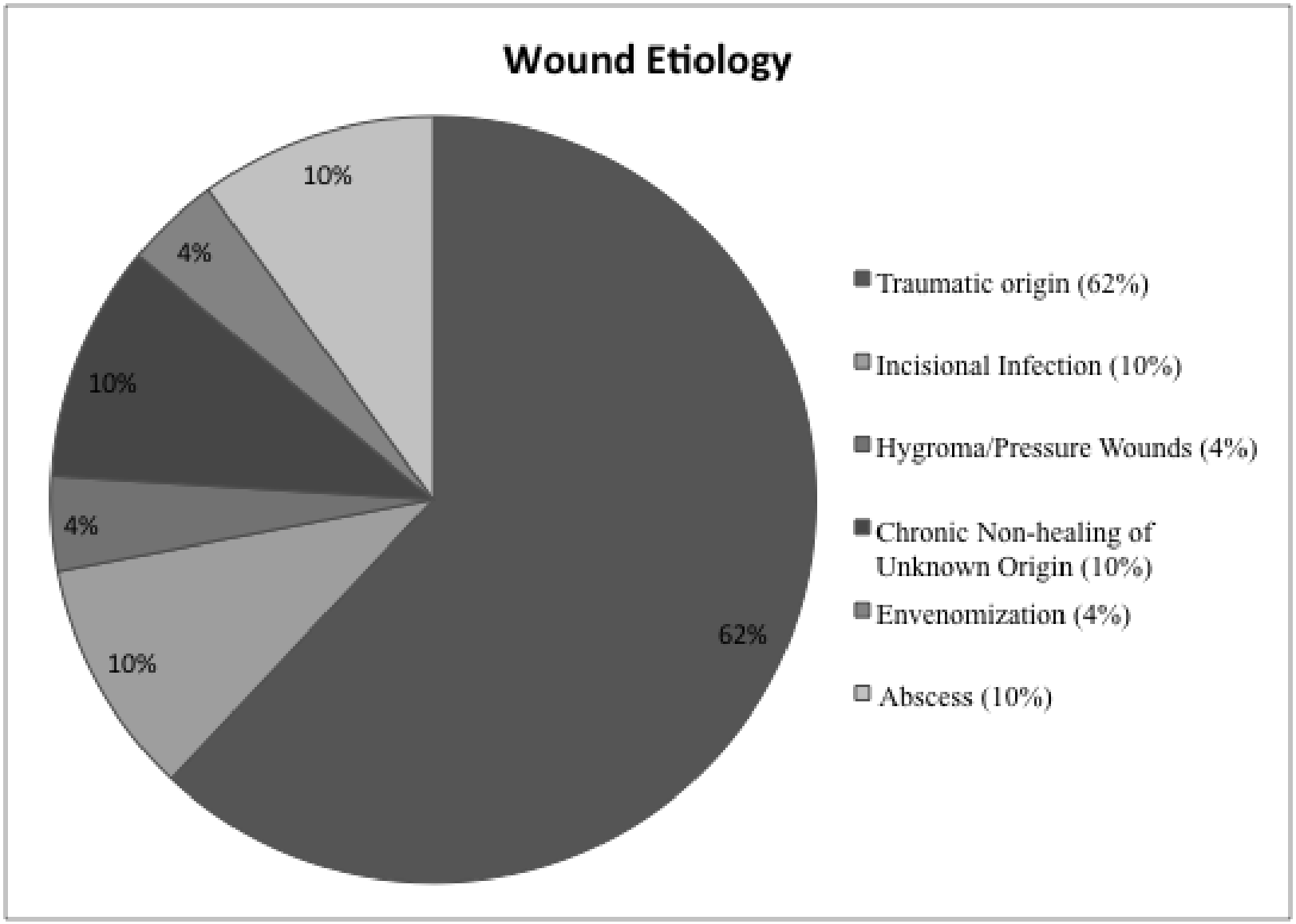


FIGURE 2.12: Pie graph showing the distribution of the 58 wounds from the 50 cases undergoing NPWT.

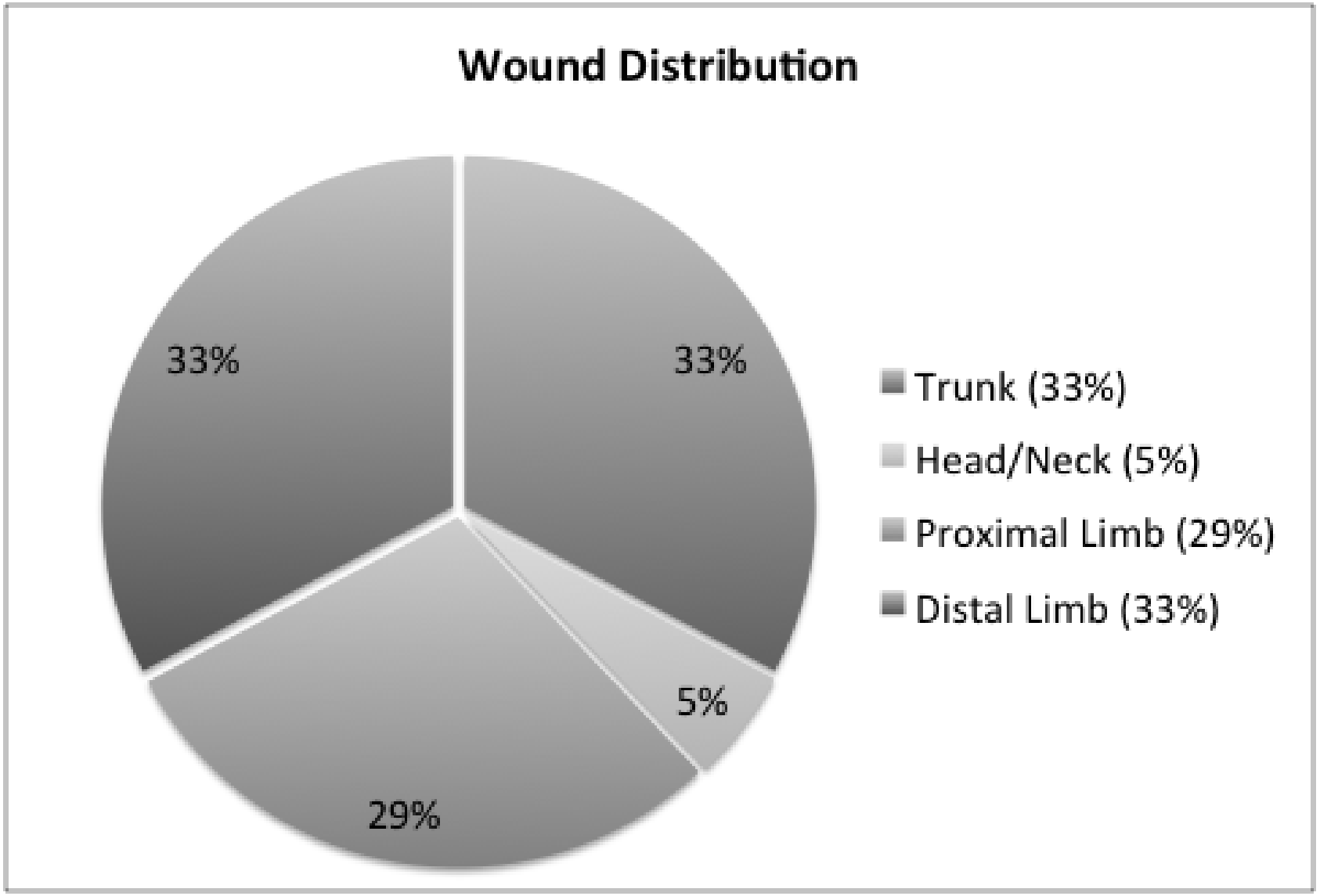
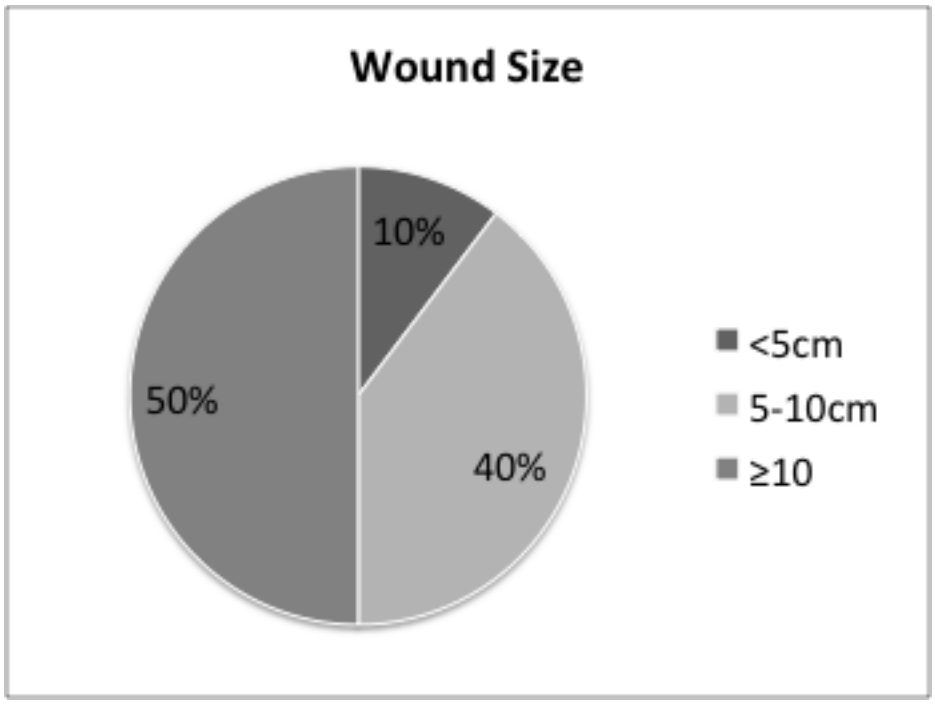


FIGURE 2.13: A) Pie graph depicting size of the 58 wounds from 50 cases undergoing NPWT.

B) Photo depicting one of the wounds on the thorax of a Labrador retriever.

A)



B)

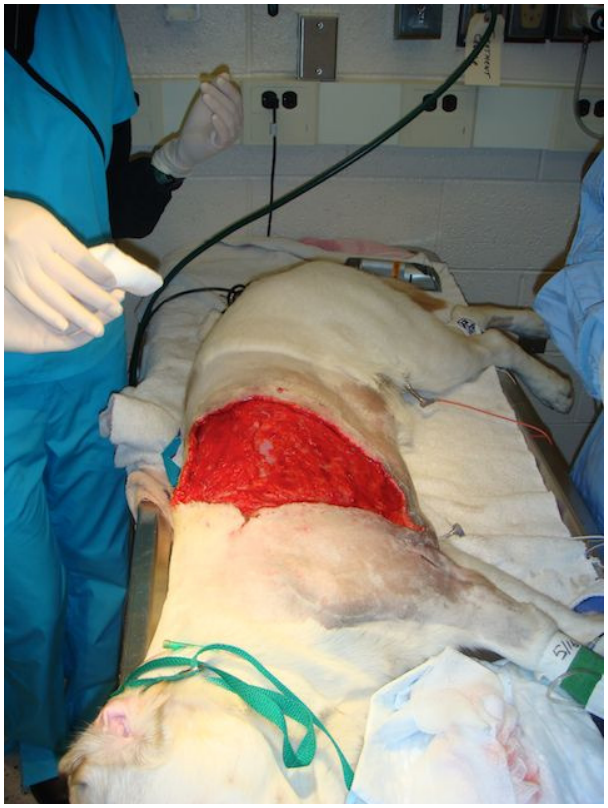


FIGURE 2.14: Graph depicting the length of time NPWT was in use for each case and the chosen primary dressing.

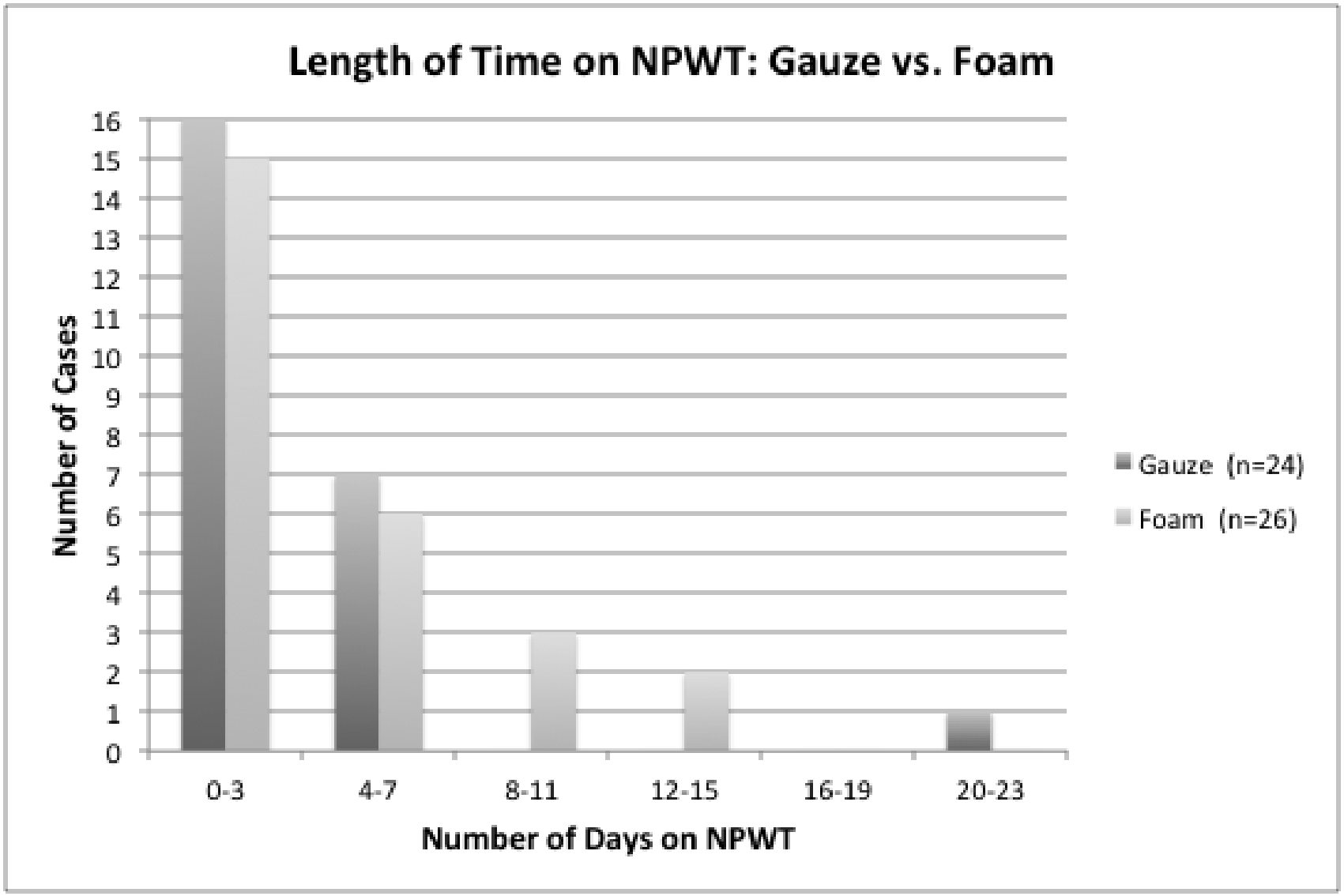
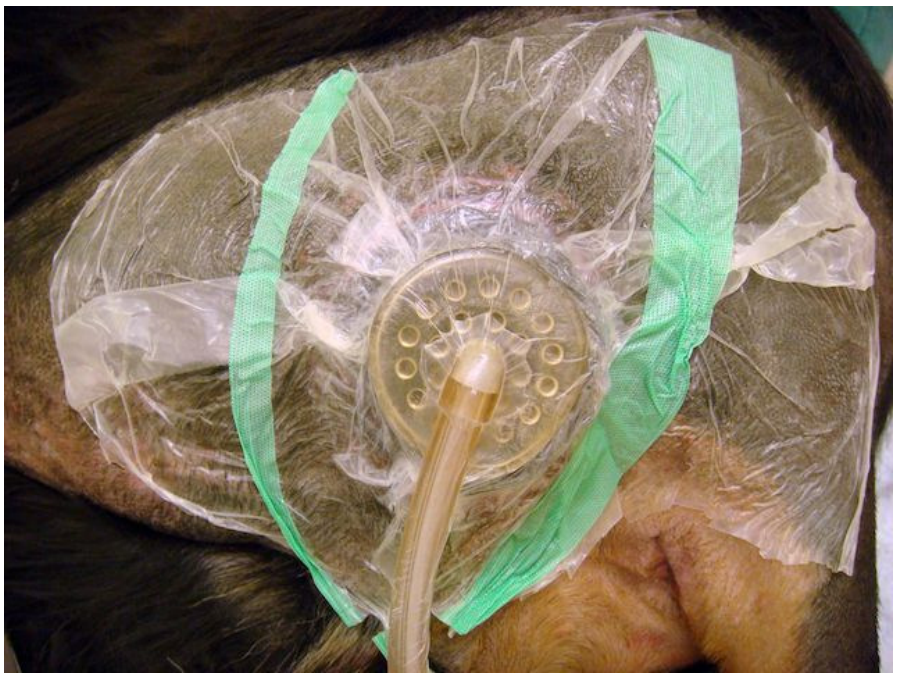


FIGURE 2.15: Periwound erythema was common when the entirety of the adhesive drape was removed at each bandage change. To minimize this, only the portion covering the wound was removed at dressing change.

A)



B)

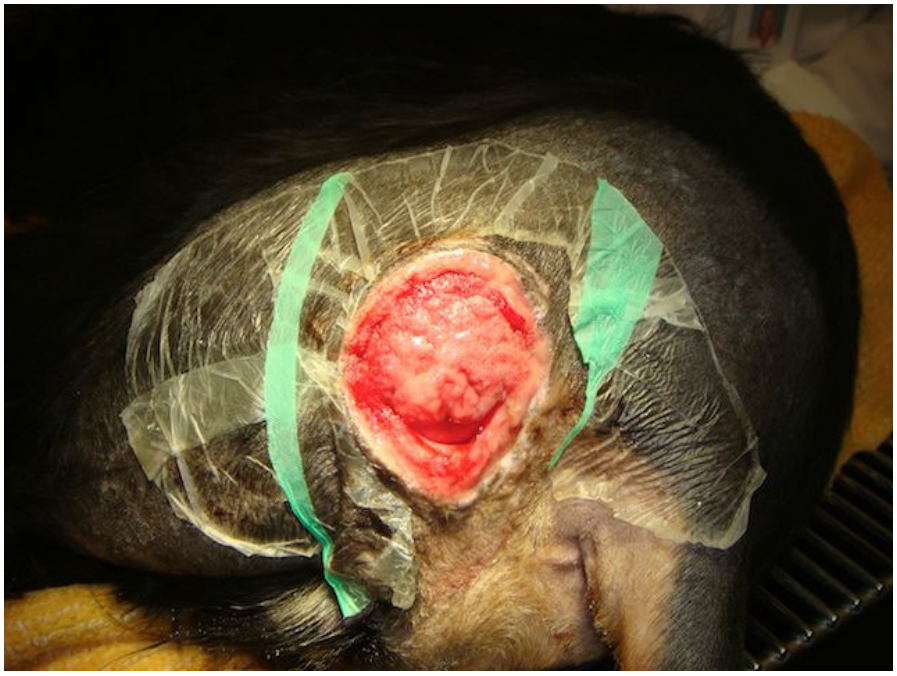
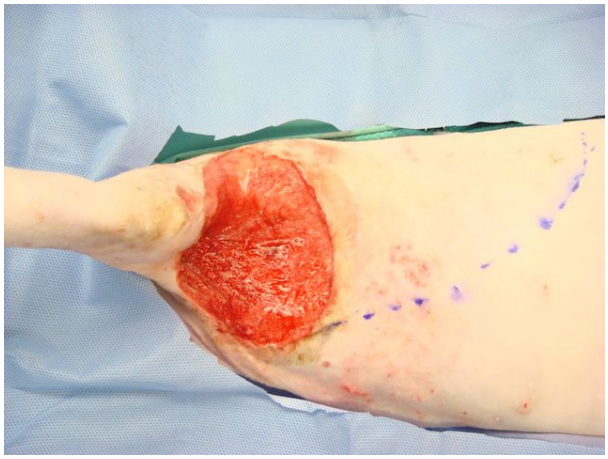
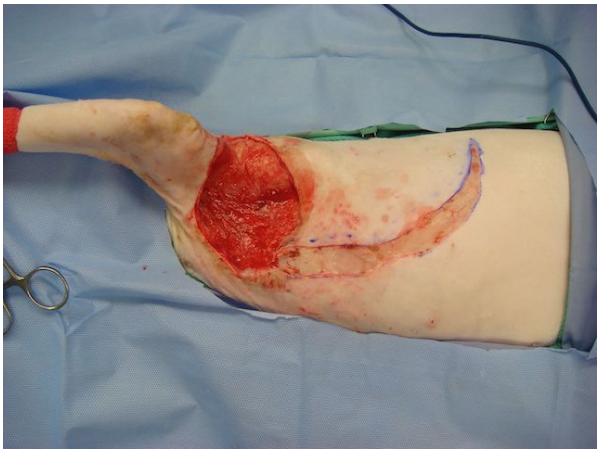


FIGURE 2.16: Early granulation appearance of granulation tissue is seen in an axillary wound of a cat after 3 days of NWPT (A). This early appearance allows for reconstructive closure. The outline for an axillary pattern flap is incised (B). The skin is then moved cranially to cover the original defect (C). The skin is then sutured into place with a simple interrupted pattern (D).

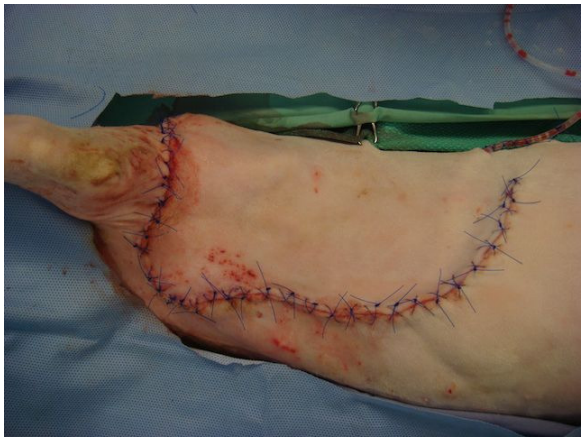
A)



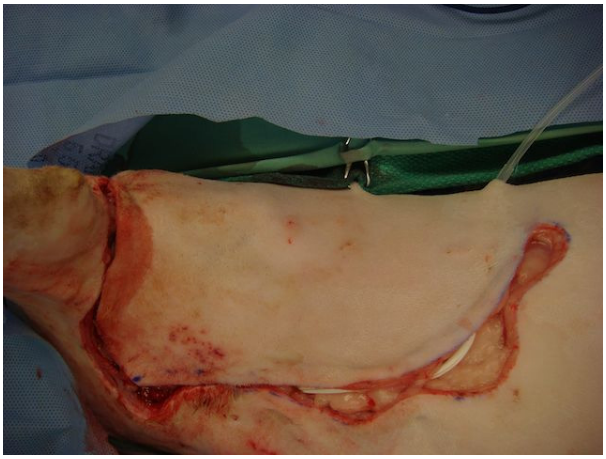
B)



C)



D)



Chapter 3: Effects of Negative Pressure Wound Therapy on Healing of Free Full-Thickness Skin Grafts in Dogs

Effects of Negative Pressure Wound Therapy on Healing of Full-Thickness Skin Grafts in Dogs

BRYDEN J. STANLEY, BVMS, MVetSc, Diplomate ACVS, KATHRYN A. PITT, DVM, CHRISTIAN D. WEDER, BS, MICHELE C. FRITZ, BS, LVT, JOE G. HAUPTMAN, DVM, MS, Diplomate ACVS, and BARBARA A. STEFICEK, DVM, PhD, Diplomate ACVP

Department of Small Animal Clinical Sciences, College of Veterinary Medicine, Michigan State University, East Lansing, MI and the Diagnostic Center for Population and Animal Health, College of Veterinary Medicine, Michigan State University, 4125 Beaumont Rd., East Lansing, MI.

This study was funded by the Michigan Animal Health Foundation, Michigan Veterinary Medical Association. Student funding was provided by NIH Grant 5T35RR017491-07 to Michigan State University.

Address correspondence to Bryden J. Stanley, Department of Small Animal Clinical Sciences, College of Veterinary Medicine, Michigan State University, East Lansing, MI 48824. E-mail: stanle32@cvm.msu.edu

Effects of Negative Pressure Wound Therapy on Healing of Full-Thickness Skin Grafts in Dogs

ABSTRACT

Objective - To compare healing of full-thickness, meshed skin grafts under negative pressure wound therapy (NPWT) with standard bolster dressings in dogs.

Study design - Randomized, controlled experimental study, paired design.

Methods - Full-thickness skin wounds (4x1.5cm) were created bilaterally on the antebrachia of 5 dogs (n = 10). Excised skin was grafted to the contralateral limb. Grafts were randomized to NPWT or standard bolster dressings (CON). NPWT was applied continuously for 7 days. Grafts were evaluated on Days 2, 4, 7, 10, 14 and 17, biopsied on Days 0, 4, 7, and 14, and cultured on Day 7. Outcome variables were: time to first appearance of granulation tissue, percent graft necrosis, and percent open mesh. Significance was set at $p<.05$. Histologic findings, culture results, and graft appearance were reported.

Results - Granulation tissue appeared earlier in the NPWT grafts compared to the CON grafts. Percent graft necrosis and remaining open mesh area were both greater in CON grafts compared to NPWT grafts at most time points. Histologic results showed no significant difference in all variables measured, and all cultures were negative.

Conclusions – Variables of graft healing were superior when NPWT was used in the first week post-grafting. Fibroplasia was enhanced, open meshes closed more rapidly and less graft necrosis occurred with NPWT application. More preclinical studies are required to evaluate histological differences.

Clinical Relevance - NPWT can be used to optimize graft survival, and may be especially valuable for large grafting procedures where immobilization is challenging.

Keywords:

Negative pressure wound therapy

Vacuum-assisted closure

Sub-atmospheric

Skin graft

Wound healing

INTRODUCTION

The full-thickness skin graft (FTSG) is the preferred type of free cutaneous graft in small animal surgery because they include the entire dermis and thus provide a hirsute, glandular and robust wound coverage following healing.[134,183] Full-thickness skin grafts are relatively easy to harvest and prepare compared to their split-thickness counterparts, requiring no specialized instrumentation such as a dermatome, and the abundance of dog and cat truncal skin generally allows the donor site to be closed directly. However, the increased thickness of the FTSG (compared to a split-thickness graft) tests the processes of graft survival. During the first 48 hours following transfer, free grafts survive by absorbing tissue fluid from the recipient bed via plasmatic imbibition and the joining of the graft and wound vessels by inosculation.[183] At around 72 hours, fragile capillary buds emerge from the recipient bed and start to vascularize the graft.[183] Graft survival depends upon successful vascular ingrowth, subsequent establishment of venous and lymphatic drainage, and the development of a fibrous (collagenous) attachment.[183,184] Causes of partial or total graft failure include fluid accumulation (seroma or hematoma) under the graft, movement of the graft, and infection.[183] Maintenance of a closely applied, immobile graft is vital to the final outcome, and should not be underestimated.

Grafts are traditionally covered with a non-adherent primary layer, and then secured firmly by a bolster dressing to prevent shear movement. The large, firm secondary layer is comprised of an absorbent wrap, cast padding and gauze wrap, acting to immobilize the graft and limb movement.[134,183] Further immobilization can be achieved by incorporating some type of device

with the tertiary bandage layer (e.g., splint, cast, external fixator). However, consistent and evenly distributed pressure can be difficult to maintain, especially in mobile and irregular areas. It is intuitive that if a dressing were capable of enhancing contact and minimizing motion between graft and recipient bed, it would result in a lower incidence of graft failures, and thus improved outcomes.

In the past decade, negative pressure wound therapy (NPWT) has become an increasingly popular adjunct in human medicine, used in a variety of wound healing and surgical applications.[73,74,185-188] Negative pressure wound therapy (also termed vacuum-assisted closure, topical negative pressure) involves the application of a regulated, sub-atmospheric pressure through a porous dressing placed over a wound bed that has been sealed from its atmospheric environment (**Figure 3.1**).[78,189,190] Experimentally, NPWT has been shown to increase blood flow to the wound, stimulate the formation of granulation tissue, and possibly reduce interstitial edema.[66,89,140,191,192] Reduction of the bacterial load in wounds with NPWT has also been documented in animal models and human patients, although findings are inconsistent.[66,94,95,131,193] Application of NPWT in human wound care is widespread, where it is employed to improve healing in soft tissue trauma, compromised flaps, open fractures, surgical dehiscence and split-thickness skin grafts.[74,84,109,110,137,145,146,148,163,179,194-198] Despite the plethora of publications documenting promising outcomes with NPWT, many surgeons remain unsure or skeptical of its effectiveness and specific indications because of the lack of randomized, controlled data in the literature.[57,73,77-79,199,200] Furthermore, with respect to skin

graft survival, skin grafting in humans is typically split-thickness, with few reports documenting the effects of NPWT on the healing of FTSGs.[201]

There are several veterinary case reports demonstrating use of NPWT in open wounds, one case series and one controlled experimental study; all suggest positive effects of NPWT.[85-88,111,170,202] In the case series reported by Ben-Amotz and Lanz et al., the use of NPWT in 15 dogs with traumatic wounds, 10 cases subsequently underwent full-thickness skin grafting and NPWT was re-applied. All grafts were reported to have survived, although percentages were not documented. To the authors' knowledge, no randomized, controlled study comparing NPWT to standard-of-care bolster dressings over FTSGs in dogs has been published.

Given the positive results with NPWT in human grafting practices, we hypothesized that the NPWT would improve healing of FTSG in dogs. Our rationale was that by immobilizing and improving contact of the graft-bed interface, by preventing fluid accumulation beneath the graft, and increasing wound blood flow, NPWT would directly enhance the plasmatic imbibition and revascularization processes. To test our hypothesis we designed a prospective, randomized, controlled, experimental study in dogs. The main objectives of this study were to compare first appearance of granulation tissue, graft necrosis and the remaining area of open meshes of FTSGs, between NPWT and standard bolster dressings at 7 time points. Secondary objectives were to report qualitative variables of graft healing at 7 time points, histological findings at 4 time points, and bacterial cultures once.

MATERIALS AND METHODS

Purpose-bred adult female beagles (n = 5) weighing between 9 and 10 kg, body condition score 4-5 out of 9, were acquired for this study. All dogs had values within normal ranges on physical examination, complete blood count and serum biochemistry performed before study commencement. There was a one week acclimatization period, during which time the dogs were conditioned to the housing, feeding and social enrichment protocols.

On day 0, dogs were medicated with acepromazine maleate (0.07 mg/kg intramuscularly [IM]) and morphine sulfate (0.66 mg/kg, IM). A saphenous intravenous (IV) catheter was placed and anesthesia was induced with thiopental (10-15 mg/kg IV to effect) and maintained on isoflurane (baseline concentration 2%) delivered in oxygen (30 ml/kg/hr). Lactated Ringer's solution (10 ml/kg/hr IV) was administered during anesthesia. Following induction, cefazolin (22 mg/kg IV once) was administered. Thoracic limbs were clipped from mid-metacarpus to just above the radio-humeral joint, the dogs positioned in dorsal recumbency, and the skin prepared for aseptic surgery.

Using strict aseptic technique, bilateral 4.0 x 1.5 cm full-thickness skin wounds were surgically created on the dorsal aspect of each mid-antebrachium (ten wounds total), using sterile templates (**Figure 3.2**). Antebrachial fascia was also excised to expose the underlying extensor carpi radialis muscle belly, which acted as the recipient bed. Hemostasis was achieved by applying pressure with gauze swabs. A 2 mm strip from a long edge of the excised skin was submitted as the Day 0 histopathology sample.

Both excised skin sections were prepared for grafting by meticulous removal of all sub-dermal remnants and eight consistent, longitudinal meshes were created using a #11 scalpel blade (**Figure 3.3).** Following preparation, grafts were transposed (i.e., right antebrachium-derived skin into left antebrachial recipient bed and *vice versa*), and secured with 20 simple interrupted sutures of 4-0 nylon (Ethilon^{TM}, Ethicon Inc., Somerville, NJ) (**Figure 3.4**). Right and left antebrachial grafts were assigned to NPWT group or control (CON) group based on computer randomization (Microsoft Excel® Random Number Generation, Microsoft Corp., Seattle, WA). Randomization assignments were only revealed following grafting to eliminate any procedural bias of the surgeon (BJS). High-resolution digital photographs (3264 x 2448 pixels) were taken at this time (Sony DSC-T200, Sony USA, New York, NY), with a carefully positioned metric scale.

A single layer of petrolatum-impregnated knitted cellulose acetate dressing (Adaptic®, Johnson & Johnson, Arlington, TX) was placed on top of the grafts in both groups. Negative pressure dressings were applied to the NPWT grafts in the following manner: skin surrounding the wound was gently wiped with a liquid medical adhesive (Mastisol, Ferndale Laboratories Inc, Ferndale, MI). An open weave gauze sponge (Venturi Dressings, Talley Medical, Hampshire, UK) was moistened with saline and contoured over the Adaptic-covered graft, including the edges. A flat, fenestrated drain was buried within this dressing and the tubing anchored to the skin with a hydrogel adhesive (Venturi Dressings, Talley Medical, Hampshire, UK). A transparent adhesive sheet (Venturi Dressings, Talley Medical, Hampshire, UK) was placed to cover the dressing, and an additional 3-5 cm border of periwound skin (**Figure 3.5**). The tubing was positioned such that it coursed proximally toward the trunk. The evacuation tubing was connected to a 3-way

stopcock, incorporated into a thoracic bandage with coiled intravenous polyurethane tubing exiting upwards. The coiled tubing went to a swiveling fixture attached to a bar above the cage (Core Flex-coil, International WIN, Kennett Square, PA), then to the canister of the NPWT therapy unit (Venturi®, Talley Medical, Hampshire, UK) mounted on the side of the cage (**Figure 3.6**).

A continuous negative pressure of -65 mmHg was selected and the dressing observed for collapse and the development of raisin-like wrinkling (indicating subatmospheric pressure) (**Figure 3.5).** The machine setting was checked against a transducer (QA-PT Parameter Tester, Metron, Grand Rapids, Michigan) at the 3-way stopcock close to the level of the dressing, every 4 -6 hours throughout the duration of NPWT. CON grafts received only the petrolatum-impregnated dressing as a primary layer. Both groups were bandaged from digits to above the elbow with identical secondary and tertiary layer bandages (Specialist Cast Padding, BSN Medical Inc., Charlotte, NC; Kendall Conform Stretch Bandage, Covidien Inc., Mansfield, MA; PetFlex, Andover Healthcare Inc, Salisbury, MA).

Before recovery from anesthesia, a second dose of morphine sulfate (0.33 mg/kg) was administered subcutaneously. Carprofen (4.4 mg/kg) was administered orally on the day before surgery and continued orally every 24 hours for 7 days. Elizabethan collars were placed on all dogs. On Days 2, 4, 7, 10, 14, and 17 all wounds underwent dressing changes, following administration of morphine (0.66 – 0.83 mg/kg IM) and acepromazine (0.07 mg/kg IM) to each dog. All dressing changes adhered to aseptic principles and antibiotics were not administered at any time during the study period. Care was taken not to disturb the grafts during these changes.

On Days 2 and 4, application of NPWT and bandaging was performed as previously described. NPWT was discontinued after Day 7, based on the rationale that factors influencing successful engraftment are most critical during the initial week following grafting, and that most grafts will haven either 'taken' or failed at that point.[183] From Day 7 on, both groups were bandaged identically until the study termination on Day 17. This bandaging consisted of a single layer of petrolatum-impregnated gauze dressing over the graft, followed by a 4x4 inch gauze, and identical secondary and tertiary layer bandages as previously described.

Dogs were housed individually in 4 x 6 ft cages, where they could interact vocally and visually with each other. They received environmental enrichment 2-3 times daily in the form of direct human contact not associated with dressing changes, usually at time of day time transducer checks. Enrichment consisted of playing, petting and grooming. Dogs were provided with selected toys in each cage. Dogs were monitored for comfort, bandage integrity and mechanical function of the NPWT machines every 4 hours during the day, and every 6 hours overnight. Consistent negative pressure at the level of the NPWT grafts was confirmed by checking that transducer readings concurred with machine settings at these time points. A negative pressure of -65 mmHg was maintained continuously until Day 4, at which time the continuous negative pressure was changed to -45 mmHg until Day 7. The pressure settings and continuous mode of NPWT were selected following consultation with the manufacturer.

High resolution digital photographs were taken at all dressing changes. The camera was angled to assume a straight, dorso-ventral view of the graft, with a metric scale carefully positioned at the same level as the graft. The grafts were not disturbed; any wound fluid or crusting was gently

removed with sterile saline-soaked gauze sponges. Subjective wound evaluations for recording qualitative variables were performed at all dressing changes (**Table 3.1**). Further to the Day 0 sample, additional graft biopsies were obtained with a 4 mm disposable dermal biopsy punch from the corner of each graft on Days 4, 7 and 14, in a systematic pattern (Day 4 proximo-medial, Day 7 disto-lateral, Day 14 proximo-lateral). Aerobic cultures were taken on Day 7 by rolling a sterile culture swab over the surface of the graft and placing it into a commercial collection and transport system (BBL^{TM} Culture Swab^{TM}; Becton, Dickinson and Company, Sparks, MA). Samples were stored at 4^{o}C and plated within 4 hours of retrieval onto 5% enriched sheep-blood agar, Columbia CNA 5% blood agar with colistin and nalidixic acid, MacConkey agar, and thioglycollate broth.

Upon study completion, all images were computer randomized and coded. Wound planimetry was performed using downloadable software (Image J Software http://rsbweb.nih.gov/ij), by an investigator blinded to the randomization and code (BJS).

Variables of graft healing

Graft healing was recorded both quantitatively and qualitatively. The first appearance of granulation tissue within the open meshes was recorded in days. The following quantitative variables were measured at each time point: 1) area of graft necrosis (cm^2); 2) total area of open mesh (i.e., not epithelialized) (cm^2); and 3) total graft area (cm^2). Necrosis was defined as black discoloration of the skin (epidermis and partial or full thickness dermis), eschar or slough of grafted epidermis or dermis. From these measurements, the percent necrosis (area $necrosis_{Day\ n}$ /

total graft area$_{Day\ n}$ x100) and the percent open mesh area (area open mesh$_{Day\ n}$/area open mesh$_{Day\ 0}$ x100) were calculated at each time point.

Qualitative assessments were made from review of the images and subjective wound evaluation forms (**Table 3.1**), for each dressing change, by an investigator blinded to the randomization (BJS). Ordinal scores of graft color were recorded at each time point, and an indication of graft mobility was obtained on Day 4. Mobility was assessed by applying gentle digital pressure on the graft, and pushing laterally, attempting to slide the graft over the bed. The presence of any seroma or hematoma beneath the graft, and bleeding from graft biopsy site was also noted.

Histologic evaluation

Samples were fixed immediately upon collection in 10% neutral buffered formalin, and processed for light microscopy. Representative sections were stained with hematoxylin and eosin (H&E) and microscopically evaluated by a board certified veterinary pathologist (BAS) who was unaware of sample grouping. Histological comparisons between groups were made at Days 0, 4, 7 and 14. The following criteria of inflammation were evaluated, and scores (in parentheses) were atttributed:

a) The concentration of neutrophilic cellular infiltration into the dermis (0-3);

b) The concentration of neutrophilic cellular infiltration into the hypodermis (0-3);

c) Edema (0-3);

d) Hemorrhage (0-3);

e) Necrosis (0-3).

The criteria used to define the concentration of cellular infiltrates were as follows: 0 = within normal histologic limits; 1 = scattered; 2 = clustered or nodular; and, 3 = diffuse. Histologic evaluation of tissue edema was based primarily on distribution within the sections, with 0= none; 1 = focal; 2 = localized (regional); and, 3 = diffuse. The degree or extent of hemorrhage within the tissue sections was subjectively and comparatively designated as: 0 = none; 1 = mild; 2 = moderate; and 3 = severe. The amount of necrosis was evaluated utilizing the following histopathologic criteria: 0 = none; 1 = focal; 2 = nodular/regional; and, 3 = diffuse (tracking along fascial planes). These 5 histologic features were weighted equally and plotted at each time point to check for any graphical interaction of each composite by group, prior to being summed to formulate an Histologic Acute Inflammation Score (HAIS; range 0-15).

Further histologic comparisons were made between groups by evaluating and scoring:

a) Epidermal Devitalization, characterized as the degree of epidermal and follicular epithelial compromise, and scored: 0 = none/normal; 1 = superficial; 2 = full epidermis; and 3 = including follicular epithelium;

b) Epidermal Hyperplasia, characterized as increase in number of keratinocyte layers when compared to Day 0 specimen sections, and scored: 0 = none/normal; 1 = mild; 2 = moderate, and 3 = severe; and

c) Neovascularization within the wound bed and hypodermis, characterized as concentration of newly forming small caliber blood vessels compared to Day 0 specimen sections, and scored: 0 = none; 1 = mild, 2 = moderate; and 3 = marked.

Bacterial evaluation

Samples were incubated and evaluated each day for quantity and species of bacteria. Bacterial isolates were to be enumerated and identified following this institution's Standard Operating Procedures for Wound Cultures. Cultures were incubated for 4 days on the Enriched Blood Agar and Thioglycollate broth, and for 2 days on Columbia CNA and MacConkey agar before considered negative.

Statistical analysis

Each dog acted as its own control, receiving both NPWT and CON. The first appearance of granulation tissue was analyzed by means of the Wilcoxon signed rank test, and presented as median and range. The quantitative response variables of Percent Area Necrosis, Percent Area Open Mesh, and Total Graft Area (cm^2) were measured 7 times (Days 0, 2, 4, 7, 10, 14, and 17). The scores of the histologic variables of HAIS, Epidermal Devitalization, Epidermal Hyperplasia and Neovascularization were measured at Days 0, 4, 7 and 14. The factors that could influence each response variable were Dog, Group (NPWT/CON), and Time. Data were analyzed by means of a three factor ANOVA with the fixed factors of Group and Time and the random factor of Dog. The errors of the data were plotted for normality; normality was accepted if the plot was unimodal and approximately symmetrical. Post hoc comparisons were by means of t-test (Group) or Bonferroni's t-test for multiple comparisons (Times vs. $Time_0$). P values are stated for all analyses.

RESULTS

All NPWT dressings and apparatus were very well tolerated for the duration of the treatment, and pressure was maintained consistently over all wounds, confirmed by the transducer readings. The maximum disparity between transducer readings and the machine settings was 4 mmHg, with an average of 1.0 mmHg. Two minor line breaks resulted in short term loss of negative pressure. These were corrected at the next integrity check by simple line replacement. Due to the frequent monitoring of the animals, we are confident that this did not affect delivery of negative pressure to the grafts for significant periods of time. No major complications were encountered on dressing changes apart from small areas of skin erythema at the flexor aspects of the elbow from bandage rub.

Quantitative Variables:

Granulation tissue was first noted in the meshes on a median of 2 days in the NPWT grafts, and 7 days in the CON grafts ($P = 0.04$) (**Table 3.2 ; Figure 3.7**).

No graft experienced catastrophic failure, but greater necrosis was noted in the CON grafts on Days 2, 4, 7 and 10, compared to the NPWT grafts ($P<0.01$) (**Figures 3.8 and 3.9**). On Days 2, 4, 7 and 10, the NPWT grafts showed less open mesh area than CON grafts ($P<0.01$) (**Figures 3.10 and 3.11**). The total graft area was not significantly different between NPWT and CON grafts at any time point, although all grafts steadily and significantly decreased in size during the study period. The mean total graft area for all grafts was 6.47 cm^2 at Day 0 and 4.46 cm^2 at Day 17, which represents a 31% reduction in total graft area.

Qualitative Variables:

Scores of graft color are presented in **Table 3.3**. All (5/5) NPWT grafts and 2 (2/5) CON grafts were non-mobile by Day 4, indicating adherence to the underlying recipient bed. One (1/5) seroma was noted under the NPWT grafts (upon biopsy); three (3/5) seromas were recorded under the CON grafts. Seromas were noted at the Day 4 or Day 7 dressing change only, usually at time of biopsy. One CON graft seroma was present at both Day 4 and Day 7. Hemorrhage from the graft biopsy site was noted in four (4/5) of the NPWT grafts and one (1/5) of the CON grafts at Day 4. On Day 7, all (5/5) NPWT and 3/5 CON grafts hemorrhaged upon biopsy. Fluid amount, color or nature of the wound fluid on dressing changes were the same between groups. Typically the wound fluid was serosanguineous, pink or brown, and of minimal quantity. By Days 14 and 17, all grafts were similar in appearance, and it was evident that the previously delineated areas of necrosis were epithelializing rapidly. Hair growth consistently appeared more robust on the NPWT grafts, compared to the CON grafts, which were less hirsute with more epithelial covering (**Figure 12**).

Histology:

There was no significant difference in the HAIS between NPWT and CON grafts (P=0.26). Histologic scores of Epidermal Devitalization, Epidermal Hyperplasia, and Neovascularization were not different between groups (P=0.54). On histologic sections, both NPWT and CON grafts showed marked epidermal devitalization at Day 4, which progressively decreased on Days 7 and 14. The epidermal necrosis presented histologically as full thickness devitalization of all keratinocyte layers, with patchy sparing of basal keratinocytes at the communication with the follicular ostia. In some areas, where epidermal architecture was still discernible, keratinocyte intracellular and intercellular edema was appreciated. Similar keratinocyte alterations extended

to involve the follicular epithelium in more severely affected tissue specimen sections. Neovascularization slowly increased at each biopsy time point, with a mean of 0/3 on Day 0, 1.5/3 on Day 7, and 2.2/3 on Day 14.

Bacterial evaluation

All aerobic bacterial cultures were negative.

DISCUSSION

Results from this small experimental study show a clear difference in measured variables of graft healing when NPWT is applied to meshed, full-thickness, skin grafts, suggesting enhanced healing when this adjunct is used, compared to standard bolster dressings. Indicators of graft healing, including first appearance of granulation tissue within the mesh interstices, and closure of the open meshes were significantly superior when NPWT was applied to the grafts. Grafts treated with NPWT showed significantly decreased necrosis compared to the CON grafts. These results show that the application of NPWT to FTSGs could be beneficial during the critical first week following grafting.

The vast majority of NPWT publications relate to the use of polyurethane open cell foam (V.A.C., Kinetic Concepts Inc., San Antonio, TX), rather than the gauze dressing used in this study. For this discussion, we have made the assumption that the mechanisms of action are similar for both types of contact dressing, based on recent studies showing equally effective delivery of negative pressure and deformation with either type.[167,168] The widely reported successes of NPWT are probably due to mechanisms of action that have been proven with *in vivo* and *in vitro* studies. These include

increased wound blood flow, promotion of angiogenesis, enhanced granulation tissue formation and stimulation of cellular proliferation and signaling pathways through the applied shear stress.[66,89,96,98,102,106,203] Other putative mechanisms of action for NPWT, such as reduction of interstitial edema, enhanced bacterial clearance, and maintenance of a moist wound healing environment have not yet been proven in basic research studies.[73,79,199] It is unknown which of these mechanisms of action, proven or otherwise, contribute most to benefit skin grafting, but one of the reasons for the improved graft healing seen in this study and suggested in the medical literature, may be in large part due to the simple immobilization of the graft when NPWT is employed.[82,109,110,146,179,197] In the early days following grafting, the graft-recipient bed interface is maintained only by a relatively weak fibrin network and whatever bolster or elastic conforming dressing is securing the site.[183] It is intuitive that the application of an evenly distributed negative pressure to press the graft firmly onto the recipient bed and increase contact area will bestow considerable benefit to the processes of graft 'take'. This contact may be further enhanced by the removal of excess fluid through the interstices of the meshes. These properties of NPWT alone would favor undisturbed growth of capillary buds from the recipient bed into the graft, and may be the reason why this modality has become so widely used in human medicine, before solid scientific proof of other mechanisms were validated.

Although increased split-thickness skin graft survival with NPWT has been widely claimed, this has not always been statistically significant, except in highly exudative grafts and when immobilization of the graft is difficult.[79,82,83,109,110] This may be due to the high overall rates of success with split-thickness skin grafting in the medical field, and the lack of randomized,

controlled, clinical trials in this area. Decreased rates of re-grafting and shorter hospitalization times have also been reported with NPWT and split-thickness grafting.[109,110,179] Although full-thickness grafting is less commonly used in human reconstructive surgery, NPWT was used both before (to prepare the wound bed) and after full-thickness grafting in a non-controlled series of 24 pediatric patients, mostly with traumatic wounds or burn contracture excisions. Mean graft take was 95% and the authors concluded that NPWT enhanced full-thickness graft take.[201]

The accelerated appearance of granulation tissue observed in this study was striking, and is consistent with what has been documented in open wounds treated with NPWT, both experimentally, and in most, but not all clinical studies in the medical literature.[66,89,111,131,186,199,204] Rapid fibroplasia is also reported in the veterinary literature, with granulation tissue appearing several days earlier in open wounds treated with NPWT, compared to standard-of-care dressings.[111] Finite element (computer) modeling has shown that the strain levels induced by applied negative pressure (5% to 20% strain) are similar to the levels known to promote cellular proliferation.[90] Experimental studies have supported this theory, showing that the deforming forces applied to the extracellular matrix and cells during negative pressure will stimulate cellular proliferation, angiogenesis and increased capillary blood flow.[98,106,191]

There was more necrosis (as evidenced by black discoloration, eschar or slough) in the CON grafts than the NPWT grafts at most time points in this study. Care was taken not to confuse the typical

early discoloration of a compromised graft with necrosis. The difference observed was greatest at Day 7, a time point at which it is generally evident whether grafted skin has 'taken' or failed. At that time, the mean percent necrosis of the NPWT grafts was < 1%, compared to 10% of the CON grafts. Although this difference was statistically significant, the difference between 1% and 10% graft necrosis may often not be considered clinically significant, especially for partial-thickness necrosis (which epithelializes well from follicular and glandular epithelium). However, the small grafts harvested and applied in this study were performed in young and healthy dogs under aseptic surgical conditions, and in an anatomical area where it is easy to apply and maintain uniform pressure. (This area was chosen to minimize the discomfort of the animals and to enable accurate planimetry). In a large, traumatic wound with an irregular wound bed, grafting can pose a significant challenge, especially so in an area difficult to immobilize. In these circumstances, the probability of partial or catastrophic graft failure is greater and the importance of optimizing graft contact even more crucial.

Mesh closure was also notably superior when NPWT was applied to the grafts, with the greatest difference again on Day 7, at the time that NPWT was removed. At that time, the average percent open meshed area in the NPWT group (36.4%) was less than half of the CON group (98.1%). When measuring mesh closure in human split-thickness meshed grafts, the medical literature refers to 're-epithelialization' of the open mesh holes.[205] In this study, which differs not only with respect to species, but also in the thickness of the grafts (full thickness) and type of meshing (by hand, compared to a mechanical mesher), it appeared to these authors that mesh closure was due to a combination of epithelialization and contraction. Mesh closure may be enhanced by the moist environment provided by the NPWT, and the lack of abrasive dressings to

disrupt the fragile migrating epithelium. The earlier appearance of granulation tissue provides a smooth bed for epithelial migration, as well as enabling myofibroblast-mediated contraction.

Qualitative variables (graft color, mobility, bleeding on biopsy, seroma/hematoma) were not analyzed statistically due to their discrete nature and low number of subjects in this study. However, these subjective changes are typically what the clinician observes during early dressing changes, and we believe they are also interesting to note in this study. Variables were different largely in the first week following grafting, and favored the NPWT grafts as evidenced by a pinker graft color, lack of mobility at Day 4, early bleeding when biopsied (indicating revascularization), and decreased fluid accumulation beneath the graft. In a more challenging clinical situation, the presence of fluid underneath the graft, and/or increased movement in those first few days could make the difference between graft healing and catastrophic failure.

One of the advantages of employing NPWT with skin grafting that has been noted in human studies is the improved quality of life immediately post-operatively. Patients receiving grafts in high-motion areas such as the neck, axilla, or perineum, require immobilization if a traditional bolster dressing is used. When NPWT is applied over the graft, these patients can have early, limited movement without compromising graft healing.[146,179] We have also found this to be applicable to dogs and cats, allowing early ambulation and easier nursing care when NPWT is applied following grafting. When possible, the graft is also bolstered with a soft padded bandage, as was done in this study.

It was interesting to note that despite the quantitative and qualitative differences seen in the variables of graft healing, there was no statistically significant difference in any of the histological scores between groups, at any time point. This lack of significance may be due to the infrequent number of biopsy time points, small numbers, or the fact that the ideal grafting conditions on the antebrachium did not challenge the treatment group enough to see a clear difference. Although not statistically significant, the mean HAIS was higher in the NPWT grafts on Day 4 (4.8 v 3.6 out of 15), and the mean Neovascularization Score was higher in the NPWT grafts on Day 4 (1.0 v 0.8 out of 3) and Day 7 (1.8 v 1.2 out of 3). It is also of interest that although assessments of follicular and glandular epidermal devitalization were similar between groups, the NPWT grafts were noticeably more hirsute than the CON grafts (**Figure 3.12**). The more robust hair growth on the NPWT grafts reflects increased viable hair follicles associated with this modality. In contrast, the sparsely haired CON grafts are consistent with more compromise to and subsequent loss of hair follicles, possibly in the superficial dermis. Further investigation of the viability of adnexal structures in grafts under NPWT is indicated.

None of the grafts in this study showed clinical signs of infection, nor was there any bacterial growth on aerobic culture on Day 7. This is likely because the wounds were created and grafted acutely under aseptic conditions. However, there was concern that if the NPWT dressings were too occlusive in nature it could increase the risk of graft maceration and subsequent infection. The role of NPWT on clearance of bacterial load from wounds remains unclear. Early investigations concluded that the application of NPWT decreased the bacterial burden of pig wounds that had been inoculated with human bacterial isolates.[66] Subsequently, several clinical studies have failed to reach the same conclusion, although it has been shown that NPWT can be

successfully employed in infected wounds that have been appropriately debrided.[84,94,95,131]

This study is not large enough to draw a conclusion concerning the effects of NPWT on contamination.

Although the numbers in this study were small, it was appropriately controlled, randomized and blinded, and statistical significance of the quantitative variables was attained. The initial study design protocol was for 10 dogs (n=20), undertaken in two series of 5 (due to the intensity of monitoring). Following analysis of results from these 5 dogs, however, the data were powerful enough to reach significance in the quantitative variables measured. This early analysis of data enabled the reduction of the total number of animals used. To identify any potential histological or bioburden differences, however, larger numbers may be required. We did not attempt to undertake any cost comparison in this study, as there was no NPWT system marketed to the veterinary profession at the time of study commencement. The initial investment for the NPWT equipment is several thousand dollars, but a true analysis would need to weigh the cost of NPWT equipment and disposables against the cost of standard dressings, plus account for the cost of any revisional procedures in either group. Such an analysis would be useful and probably be best performed as part of a prospective clinical study.

The results of this study validate the use of NPWT over full-thickness skin grafts in clinical veterinary medicine, and this modality has become the standard-of-care when performing FTSGs in our teaching hospital. Further studies are now indicated in the form of randomized, controlled prospective clinical trials, to provide the most rigorous data. No such studies exist in veterinary medicine with respect to NPWT. Additional experimental veterinary investigations into this

modality are also encouraged, refining its application, focusing on other potential indications, and evaluating overall cost-effectiveness.

Acknowledgements:

Eric Zellner, Humphrey Petersen-Jones, Duncan Petersen-Jones. The authors thank the owners who adopted the dogs into private homes at the conclusion of the study.

APPENDIX C

Tables and Figures Relevant to Chapter 3

TABLE 3.1: Form for subjective evaluation of grafts. Following study completion the forms were randomized and analyzed without revealing the grouping (NPWT or CON).

Dog ID:				Right		Left
Day: 0____2____4____7____10____14____17____						
Fluid amount	None		Minimal		Moderate	Excessive
Fluid color	Clear		Pink/Red	Brown	Yellow	Green
Nature of fluid	Serous	Serosanguineous	Sanguineous	Purulent +	Purulent ++	Purulent +++
Appearance of secondary layer		Dry & clean	Dry & stained	Moist	Wet	Strike-through
Graft adherence Day 4		Non-mobile			Mobile	
Graft color	1= Healthy pink	2= Mottled pink	3= Mottled, purple	4= Dark purple/black	5= Slimy white	
Notes on graft color:						
Periwound	Normal	Edema	Erythema	Ulceration	Induration	Discoloration
Graft hydration	Normal	Maceration +	Maceration ++	Maceration +++	Desiccation +	Dessication ++
Additional notes:						

TABLE 3.2: Table showing day of the first appearance of granulation tissue in the interstices of the meshed grafts in dogs.

Dog ID	NPWT	CON
F	2	10
G	4	10
H	2	7
I	2	4
J	4	7

TABLE 3.3: Mean results of graft color of each group. 1= healthy pink; 2 = mottled pink; 3 = mottled, bruised; 4 = dark purple or black; 5 = slimy white.

	Day 0	Day 2	Day 4	Day 7	Day 10	Day 14	Day 17
NPWT	1	1.8	2.2	1	1	1	1
CON	1	2.6	3.0	2.2	1.2	1	1

FIGURE 3.1: Application of negative pressure wound therapy, showing open cell foam (A) or gauze (B) as contact layer through which subatmospheric pressure is applied, once the area has been sealed with an impermeable, adhesive drape. As the negative pressure is applied, the dressing takes on a hard and wrinkled appearance.

A)

B)

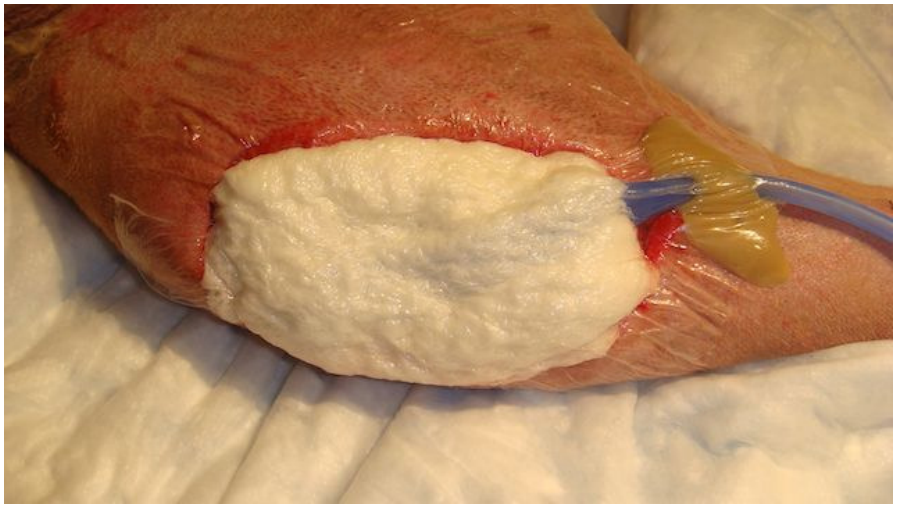


FIGURE 3.2: Dorsal, mid-antebrachial wounds were created bilaterally on using 4.0 x 1.5cm sterile templates.

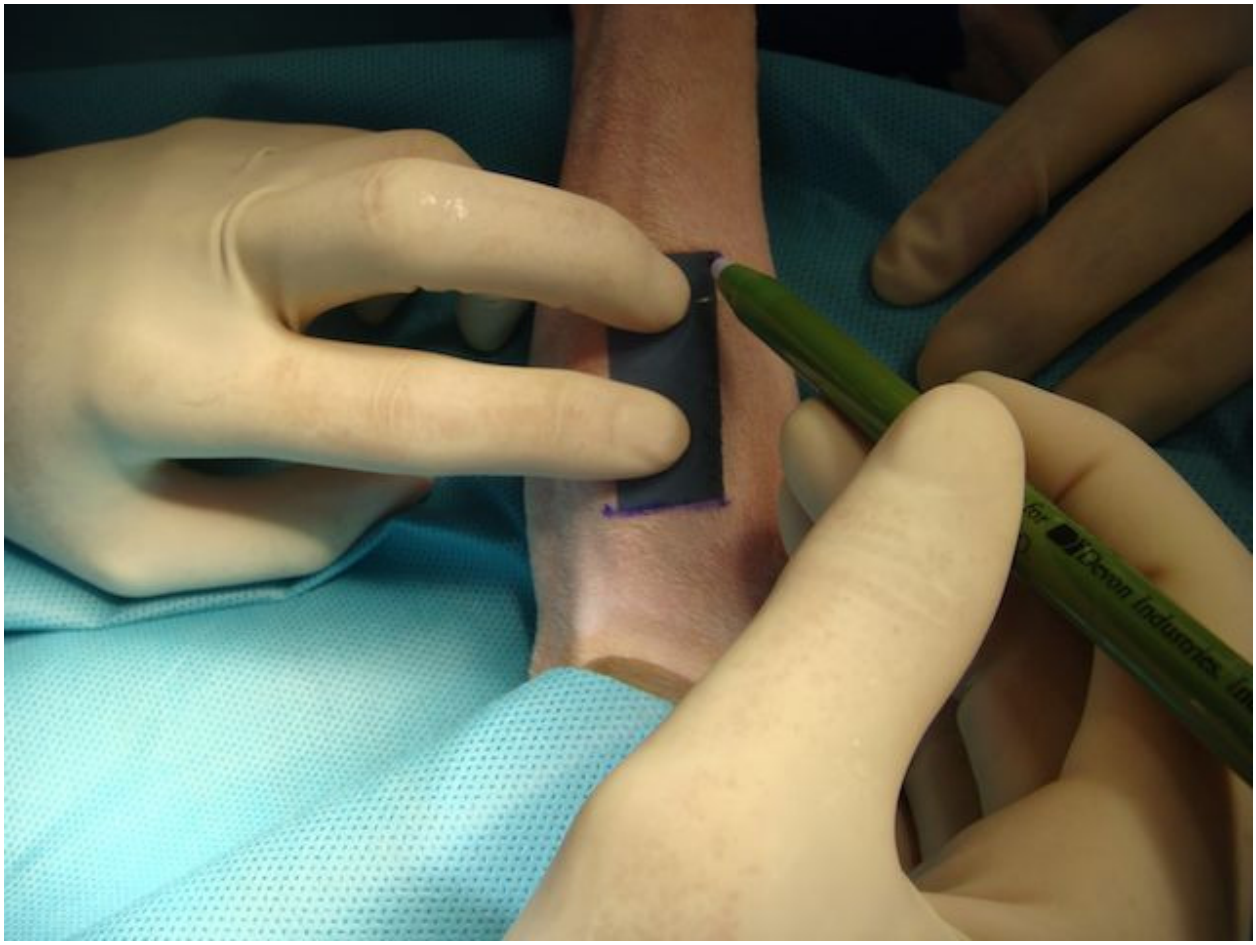
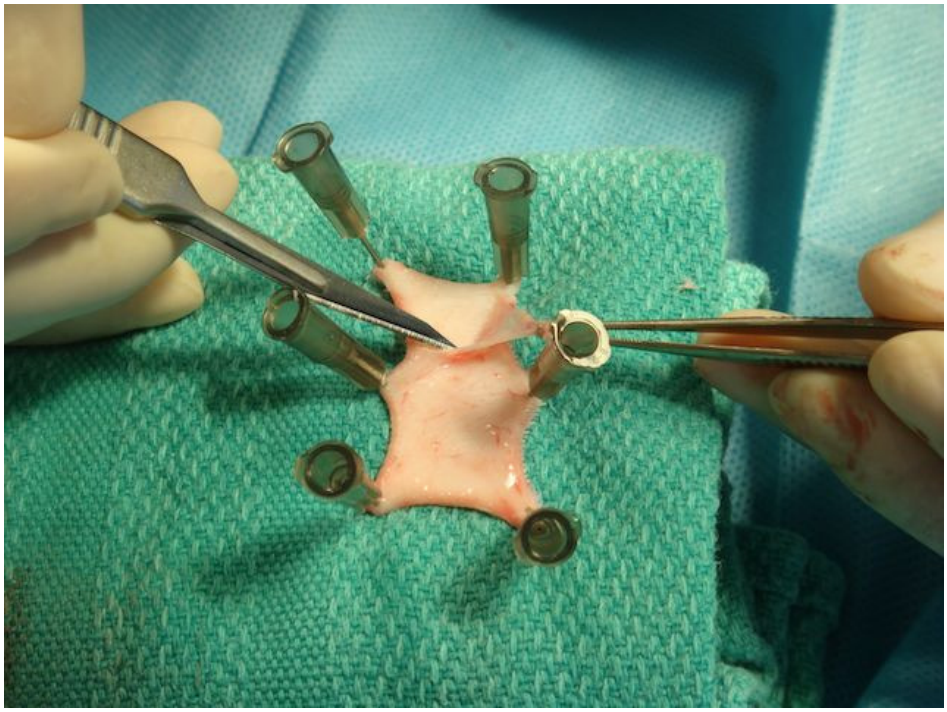


FIGURE 3.3: The excised skin sections were prepared for grafting by meticulous removal of hypodermal remnants with #11 scalpel blade (A), followed by meshing (B).

A)



B)

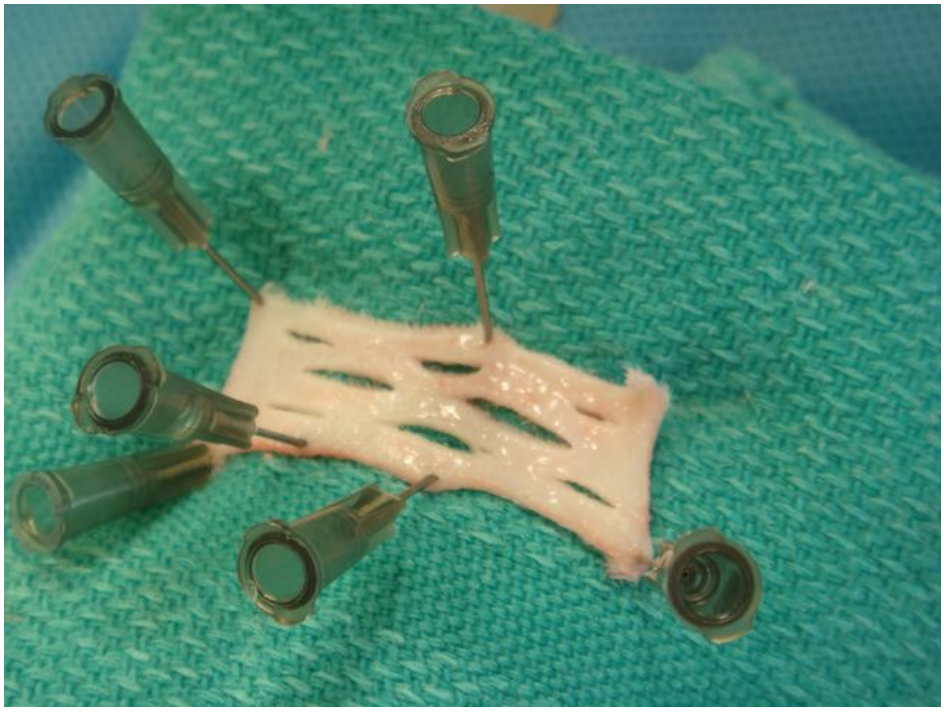


FIGURE 3.4: Graft transposed and secured in the contralateral recipient defect.

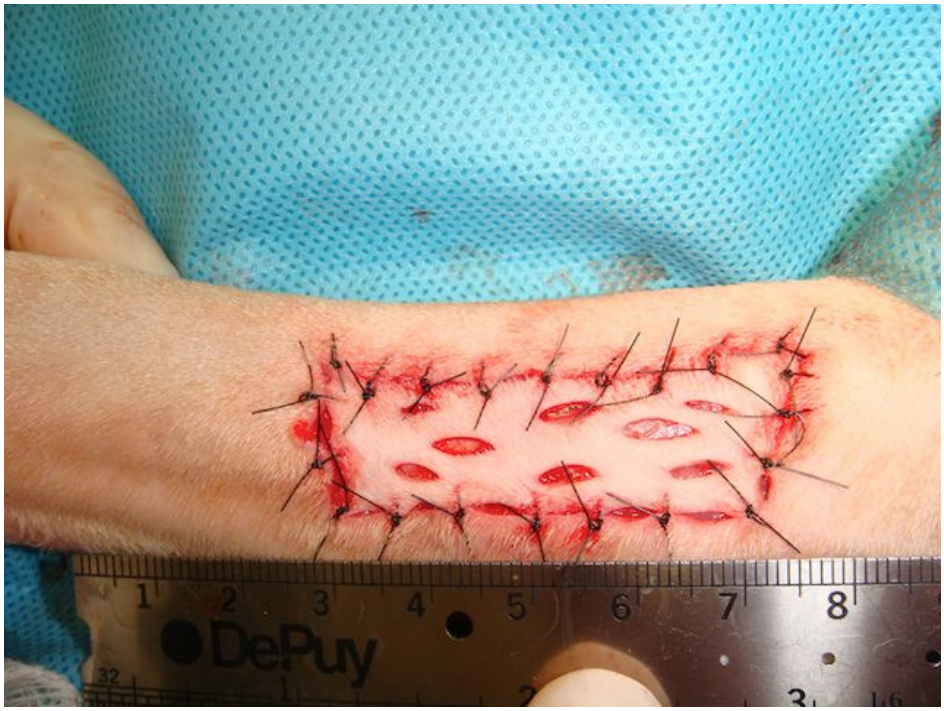


FIGURE 3.5: NPWT dressing in place before the secondary and tertiary layer bandaging. Note the hard, wrinkled appearance of the dressing, indicating that a vacuum has been obtained.

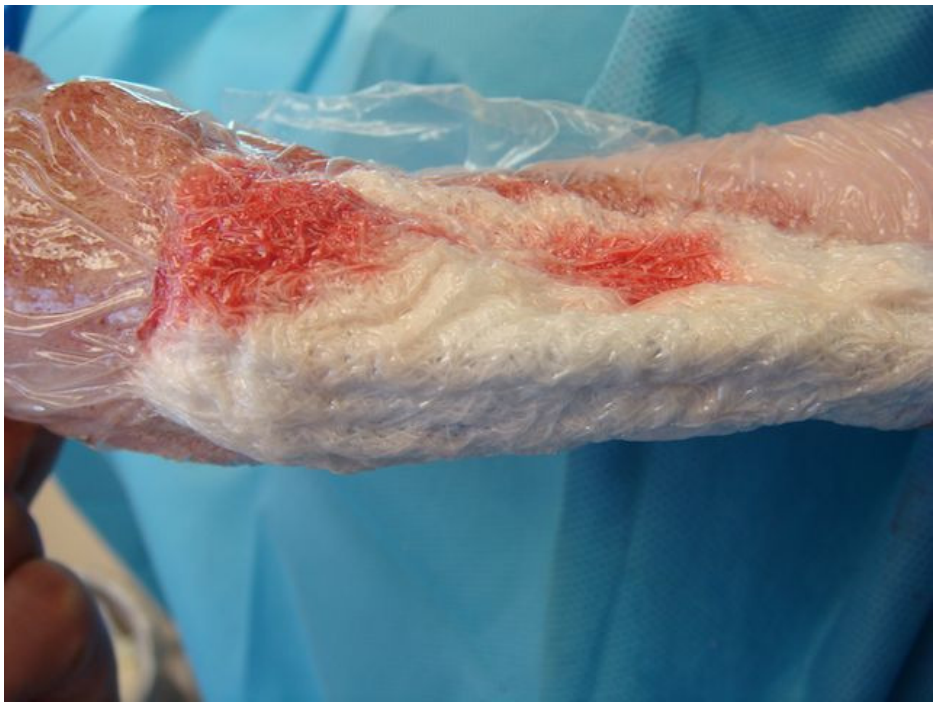


FIGURE 3.6: The negative suction tubing was secured around the thorax and exited dorsally through the coiled extension tubing to a connecting bar overhead. The NPWT machine can be seen secured to the wall on the left hand side of the cage. This dog is wearing an E-collar, and a baby “onesie” over the limb and thoracic bandage.

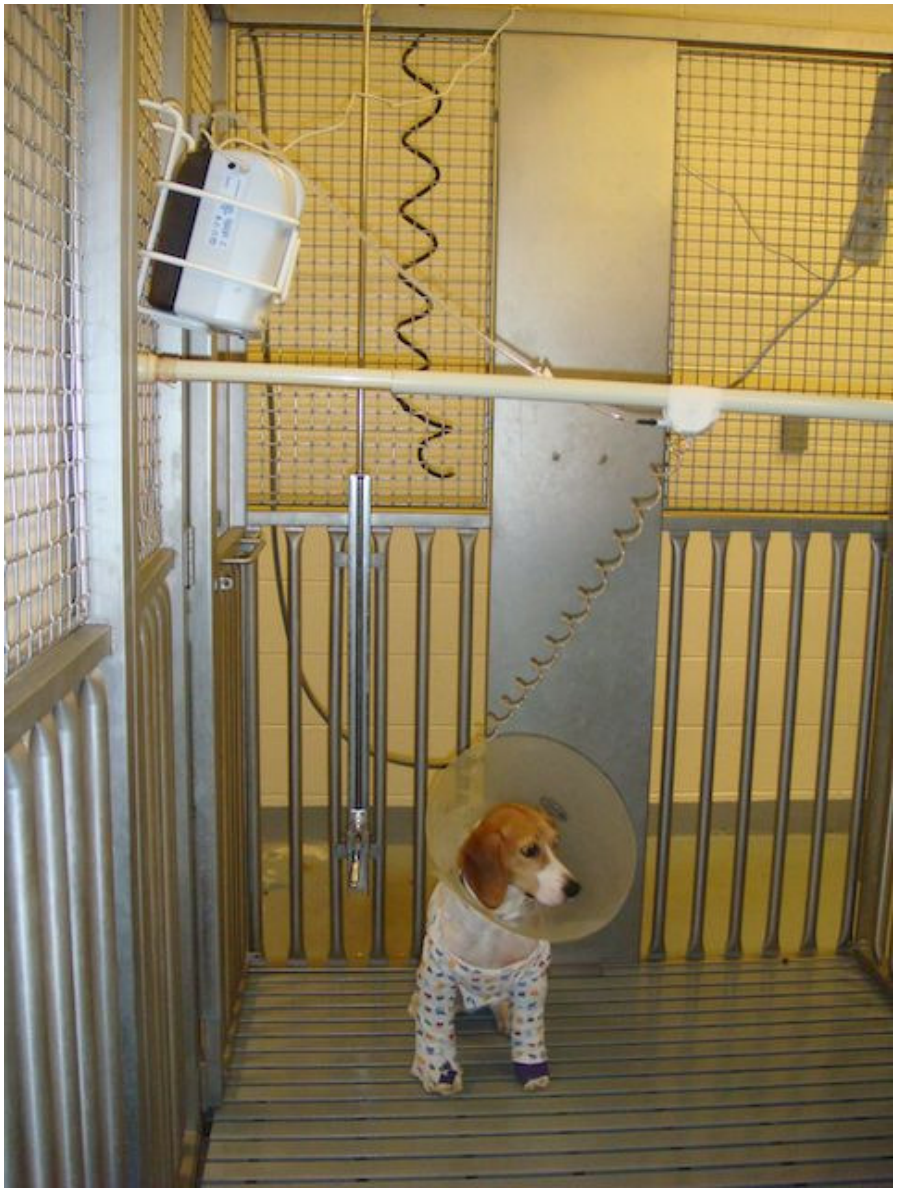
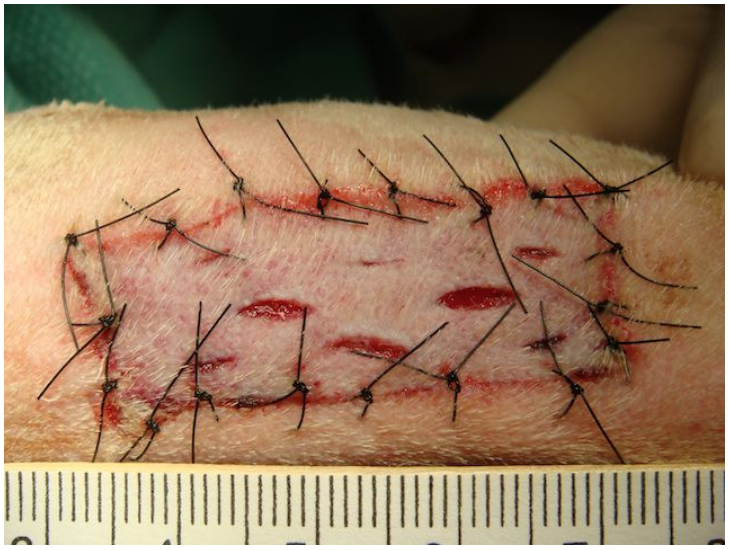


FIGURE 3.7: Typical appearance of paired grafts at Day 2. Bright red granulation tissue is seen within the interstices of the meshes in the NPWT graft (A). In contrast, the muscle belly is still visible within the meshes of the CON graft (B) . Note also the discoloration of the grafts: the NPWT graft (A) appears to be a healthy pink, compared to the more typical darker, mottled appearance of the CON graft (B).

A)



B)

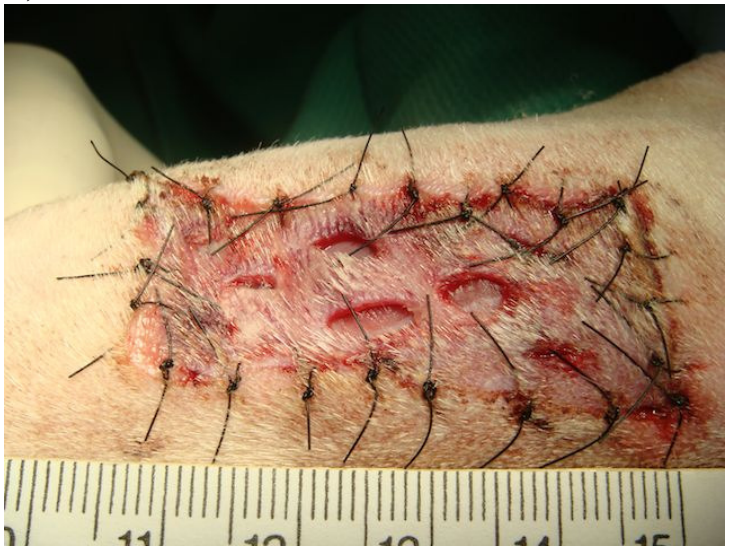
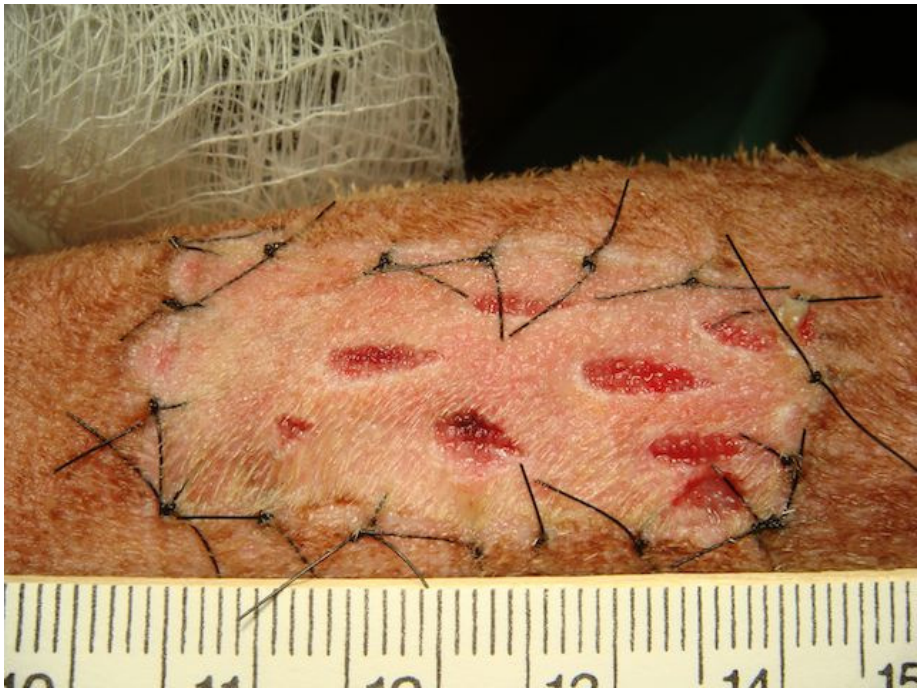


FIGURE 3.8: Typical appearance NPWT graft (A) and CON graft (B) in the same dog at Day 7, showing more compromise and partial necrosis in the CON graft.

A)



B)

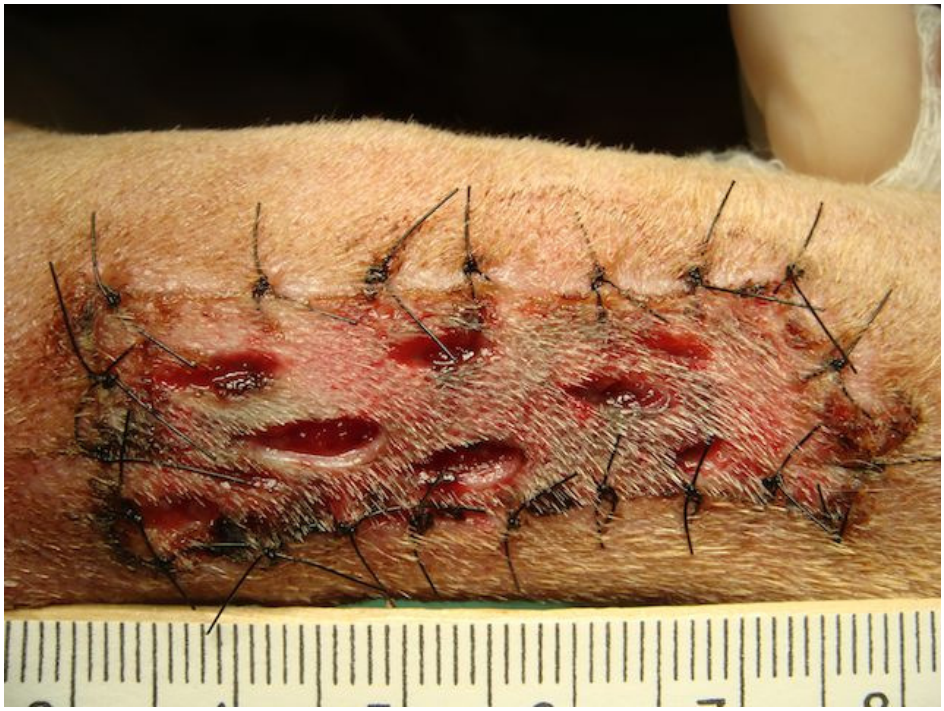


FIGURE 3.9: Mean percent necrosis of meshed FTSGs under NPWT and standard-of-care bolster dressings (CON) (n=10). Standard error bars are shown; asterisks indicate significance.

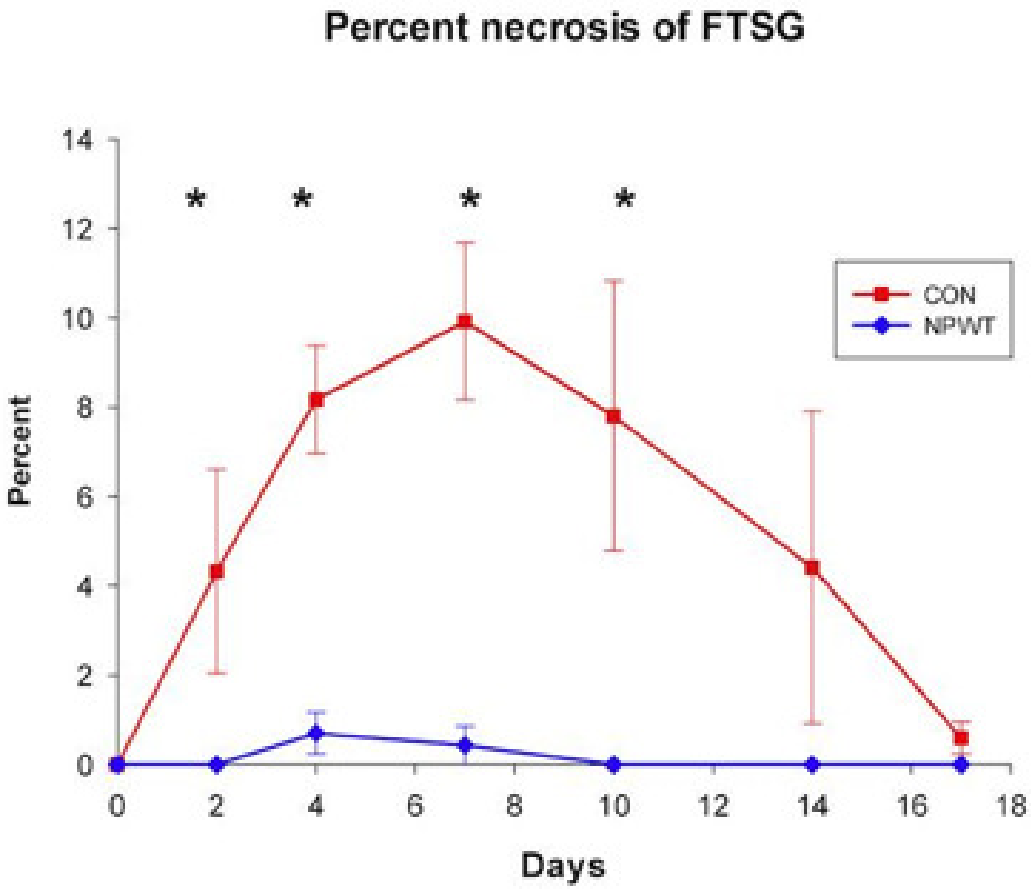
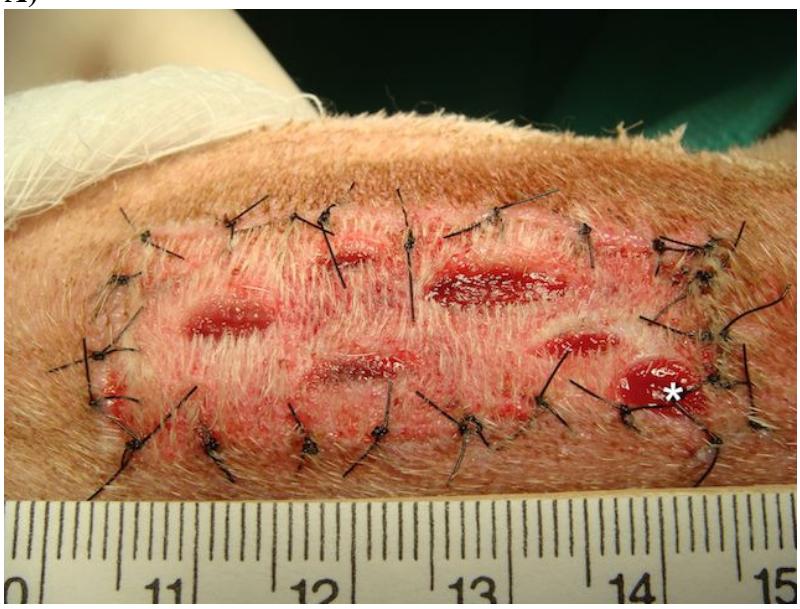


FIGURE 3.10: Typical appearance of paired grafts in the same dog at Day 7. Note the smaller size of the open meshes in the NPWT graft (A) compared to the CON graft (B). (Biopsy sites are marked with an asterisk).

A)



B)

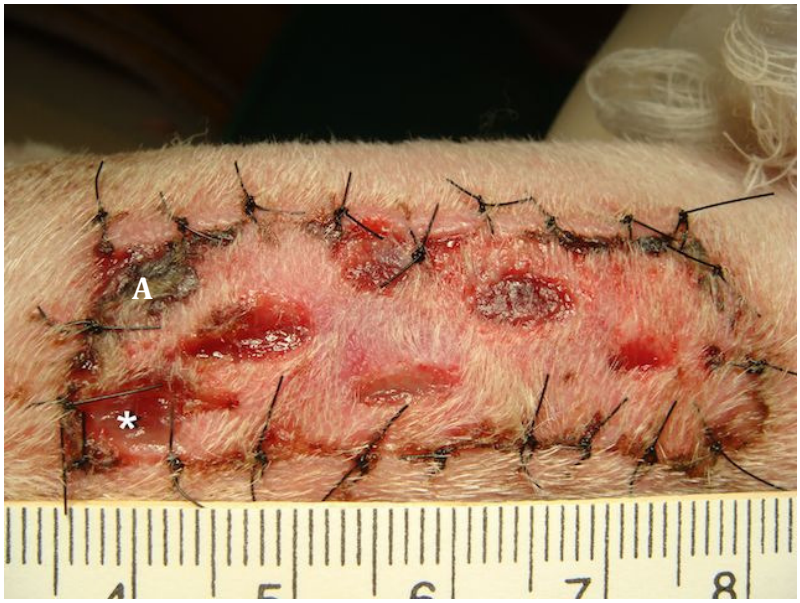


FIGURE 3.11: Mean percent open mesh area of meshed FTSGs under NPWT and standard-of-care bolster dressings (CON) (n=10). Standard error bars are shown; asterisks indicate significance.

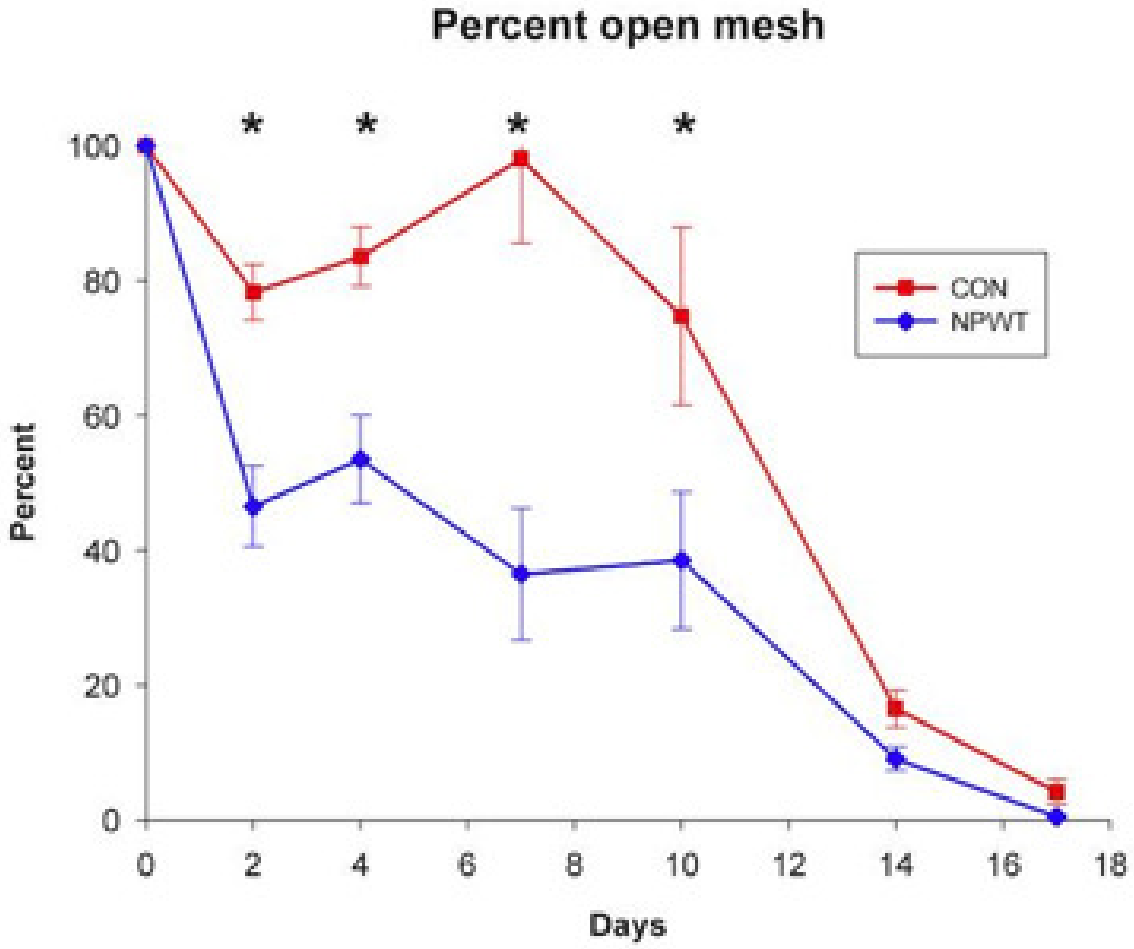
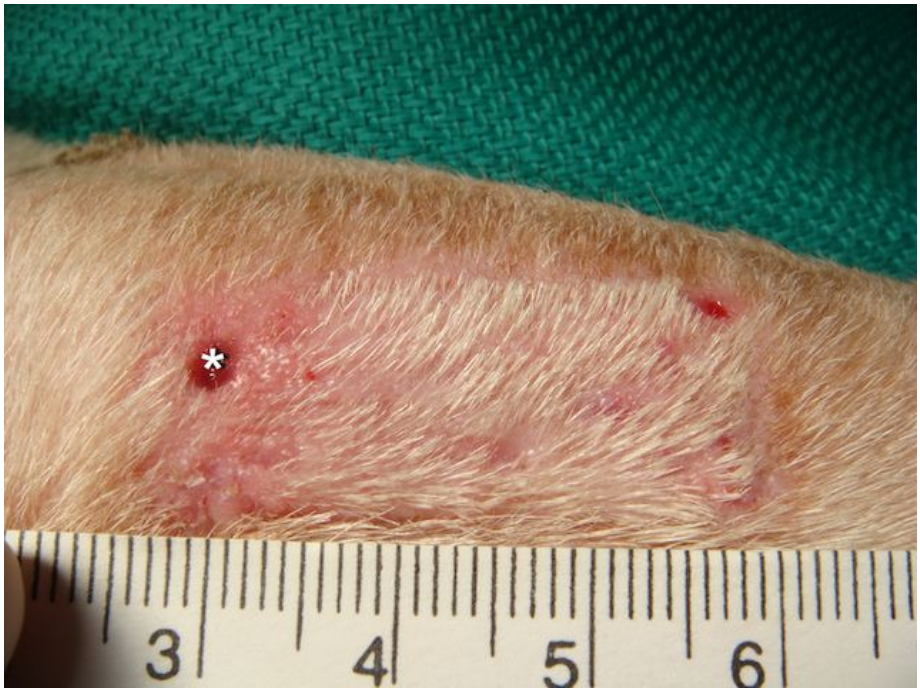
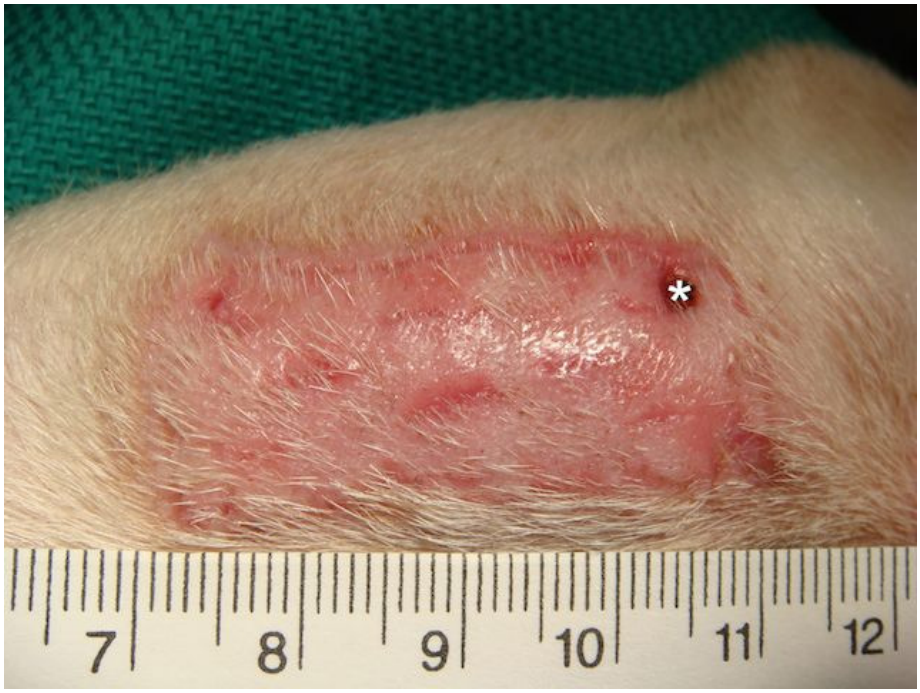


FIGURE 3.12: Typical appearance of both NPWT grafts (A) and CON grafts (B) at Day 17. Note the superior hair growth on the NPWT graft. Biopsy sites are marked with an asterisk.

A)



B)



APPENDIX D

Formal Permission to Reprint Article

Figure 3.13: Email correspondence indicating formal approval to reprint article

Mail Properties

From:	Permission Requests - UK <permissionsuk@wiley.com>	Thursday - April 26, 2012 4:12 AM
To:	"pittkath@cvm.msu.edu" <pittkath@cvm.msu.edu>	
Subject:	FW: Veterinary Surgery Reprint Authorization	
Attachments:	Reprint Authorization.pdf (63 KB)	[View] [Open] [Save As]
	Mime.822 (221 KB)	[View] [Save As]

Dear Kathryn A Pitt,

Thank you for your request.

As the author of the original Wiley material, you may reuse your article in your thesis subject to the usual acknowledgements without the need to acquire formal permission.

However, if you do wish to obtain a free permissions license, please submit your request to RightsLink, our automated permissions service.

Simply follow the steps below to obtain permission via the RightsLink® system:

* Locate your desired content on the Wiley Online Library website (http://onlinelibrary.wiley.com/)
* Navigate to the abstract page:

http://onlinelibrary.wiley.com/doi/10.1002/14651858.CD009261.pub2/abstract

* Click on the 'Request Permissions' link, to the right of the journal image
* Follow the online instructions and select your requirements from the drop down options
* Click on 'quick price' to get a quote
* Create a RightsLink® account to complete your transaction (and pay, where applicable)
* Read and accept our Terms & Conditions and download your license
* For any technical queries please contact customercare@copyright.com<mailto:customercare@copyright.com>

Kind Regards
Emma Willcox

Permissions Assistant
John Wiley & Sons Ltd.

From: Kathryn Pitt [mailto:pittkath@cvm.msu.edu]<mailto:[mailto:pittkath@cvm.msu.edu]>
Sent: Friday, April 20, 2012 7:05 PM
To: Supple-Pollard, Lydia - Boston
Subject: Veterinary Surgery Reprint Authorization

Dear Lydia Supple-Pollard,

My name is Kathryn A Pitt and I am completing a Masters Thesis at Michigan State University entitled "Negative Pressure Wound Therapy: Help or Hassle?" I would like your permission to reprint the following in my thesis: "Effects of Negative Pressure Wound Therapy on Healing of Full-Thickness Skin Grafts in Dogs" submitted to Veterinary Surgery by Bryden J. Stanley, BVMS, MVetSc, Diplomate ACVS, Kathryn A. Pitt, DVM, Christian D. Weder, BS, Michele C. Fritz, BS, LVT, JOE G. Hauptman, DVM, MS, Diplomate ACVS, and BARBARA A. STEFICEK, DVM, PhD, Diplomate ACVP. This article has been accepted for publication by Veterinary Surgery but has not yet been put in print. If you are not the individual I should be contacting about this issue please let me know.

The requested permission extends to any future revisions and editions of my thesis, including non-exclusive world rights in all languages, and to the prospective publication of my thesis by ProQuest Information and Learning (ProQuest) through its UMI® Dissertation Publishing business. ProQuest may produce and sell copies of my thesis on demand and may make my thesis available for free internet download at my request. These rights will in no way restrict republication of the material in any other form by you or by others authorized by you. Your signing of this letter will also confirm that you own [or your company owns] the copyright to the above-described material.

If these arrangements meet with your approval, please sign the attached letter where indicated and return it via Fax: ((517) 432-4091 attn: Dr. Bryden Stanley) or Email:pittkath@msu.edu if possible.

Thanks you for your time.
Sincerely,
Kathryn A Pitt

Chapter 4: Where do we go from here?

INTRODUCTION:

Further research needs to be done on the mechanisms of action and clinical applications of NPWT. Veterinary publications are limited to case reports,[85,87,88,169-171,202] case series,[86] with one controlled, experimental study.[111] Overall, the literature regarding NPWT is lacking in randomized, controlled studies evaluating outcomes of NPWT compared to standard-of-care, and there are insufficient data clarifying its mechanisms of action. Due to the testimonial and case report evidence, clinical penetration of this modality has clearly preceded scientific validation required to elucidate the physics and physiology of its efficacy, as well as refine its applications. Because of this lack of randomized, controlled studies many surgeons remain unsure or skeptical of its effectiveness and specific indications.[77,79,199,200,206-213]

The Wound Healing and Management (WHAM) Laboratory at Michigan State University headed by Dr. Bryden Stanley has been working hard to fill the literature gap in veterinary medicine. In a recent study, our group demonstrated that granulation tissue appeared significantly earlier in open wounds in dogs when NPWT was applied, and was smoother and less exuberant than the control wounds.[111] However, NPWT appeared to impede contraction and epithelialization when applied for longer than 10 days. Another controlled study in dogs, completed as part of this thesis and detailed in Chapter 3, compared the acceptance of full-thickness skin grafts with NPWT to

standard-of-care dressings.[1] This study also showed that NPWT promoted the earlier appearance of granulation tissue, as well as superior mesh healing, and decreased graft necrosis with NPWT. Although these investigations have been conducted as veterinary studies, they have been recognized as relevant to human wound care medicine, due to their similar wound healing processes which are common to all mammals.

The WHAM Laboratory (Stanley, Pitt) at the College of Veterinary Medicine has been successful in securing collaboration with the College of Human Medicine (Drs Nigliazzo, Basson, and Saxe) to continue its quest to add controlled clinical studies to the NPWT literature, and is now investigating the effects of NPWT on the healing of closed incisions. The overall goal of this project is to determine if the application of NPWT will enhance the healing of closed incisions, especially addressing any increases obtained in tensile strength of the incision. Closures under tension are performed frequently in veterinary medicine, and carry increased risk of dehiscence.[214] Major goals of cutaneous reconstruction (in any species) are to reduce tension and shear forces on the suture line. Currently, protection of suture lines under tension is obtained by performing additional surgical procedures such as pretensioning, extensive undermining, tension-relieving sutures, relaxing incisions, and skin flaps. Patients will also commonly have external coaptation applied, drains inserted, and strict activity restriction enforced. Failure to address excessive tension and shear forces results in wound dehiscence, frequently requiring re-operation, higher

[1] Effects of Negative Pressure Wound Therapy on Acceptance of Free Full-Thickness Skin Grafts in Dogs, manuscript accepted 02-07-2012, VSU-11-147. Included as Chapter 3 in this thesis

morbidity, longer hospitalization times, and increased cost of treatment. The advantages of an application that could mitigate the disruptive forces acting on the incision *and* enhance the processes of wound healing would be considerable. It would lead to higher success rates, fewer revisional procedures, decreased patient morbidity, shortened hospitalization times and reduced owner costs.

HYPOTHESIS:

Our central hypothesis is that the application of NPWT over closed incisions will accelerate healing by both reducing disruptive forces on the incision and by promoting early fibroplasia. This hypothesis has been formulated from data amassed from our veterinary clinical cases, experimental data, as well as recent clinical reports and finite element analyses.[125,127,215] More specifically, we propose that the application of NPWT over a closed incision will a) reduce tension and shear on the wound edges by immobilizing and splinting the incision, and drawing the wound edges together, and b) promote the early appearance of granulation tissue across the incisional wound gap, thus increasing tensile strength of closed incisions.

DETAILED METHODS:

Female dogs (n=30) presenting to the MSU Veterinary Teaching Hospital (VTH) for routine ovariohysterectomy will be enrolled with owner informed consent, upon meeting entry criteria (healthy physical examination, 0.5-2 yrs, >10 kg, no known medical issues, complete blood count and serum biochemistry within normal values). In addition to Institutional Animal Care and Use Committee (IACUC) approval, VTH Clinical Study approval will be obtained prior to study commencement. Dogs will be prepared for surgery per standard VTH protocol. A 16 cm, fusiform shape will be drawn along the ventral midline from the umbilicus caudally using a

template, and premeasured points along the shape will be marked with semi-permanent marker (**Figure 4.1**). Incisions will be made along the lines.

Standardized ovariohysterectomies will be performed, and the incisions routinely closed in three layers – body wall (2-0 polydioxanone, simple continuous), deep subcutaneous layer (2-0 polydioxanone), and 15 simple interrupted skin sutures, 1 cm apart, 5 mm from edge (3-0 polypropylene). The incisions are not considered to be at increased risk of clinical failure. The incision will be digitally photographed[2] with a metric measurement scale before dressing application. To avoid surgeon bias, dogs will be randomized[3] only after the incisions have been sutured. Incisions will receive NPWT (n=15=VAC), or standard dressings (n=15=CON). The system utilized to apply NPWT will be VAC® Freedom Units.[4] The VAC group will have NPWT applied according to manufacturer's recommendations, at -125 mm Hg, continuous pressure. The CON group will have a non-adherent semi-permeable primary adhesive dressing.[5] All dogs will have a light abdominal wrap bandage placed,[6] and placed in the VTH Nursing Care Unit, where the dogs will be continually monitored for comfort, bandage integrity, and

[2] Sony DSC-T100, extra-macro setting, extra-fine resolution, standardized lighting protocol

[3] Microsoft Excel® Random Number Generation, Microsoft Corp., Seattle, WA

[4] Kinetic Concepts Inc., San Antonio, TX

[5] Telfa Island Dressing, Kendall Covidien, Mansfield, MA

[6] Specialist Cast Padding, BSN Medical Inc., Charlotte, NC; Kendall Conform Stretch Bandage, Covidien, Mansfield, MA; PetFlex, Andover Healthcare Inc., Salisbury, MA.

mechanical function of the V.A.C.® system. If negative pressure is lost, the dressing will be changed. On Day 4, the dogs will be re-anesthetized and the dressings will be removed and incisions photographed. Sutures 1-3, 7-9 and 13-15 will be removed and the tensiometer attached by fine skin hook at the 3 marked points, in a randomized order (**Figure 4.2**). Distraction of the incisional skin edges and force measurement will be performed using a uniaxial electromechanical testing system operated by an investigator blinded to the grouping (VAC or CON). The data will be digitally recorded as a standard continuous stress/strain curve with a discrete peak force data point captured immediately before gross separation of the skin edges. The testing system will employ a linear drive motor with a constant distraction rate that is yet to be determined. An integrated mechanical safety device will prevent additional opening of the wound edges beyond that needed to capture the study data.

Following measurements, biopsies will be performed as shown in **Figure 4.2**. Incisions will be re-closed with skin staples. Dogs will be discharged to owners and re-checked at 10 and 20 days, photographed, and the scars scored using a veterinary-modified scar scale by an investigator blinded as to the grouping (**Table 4.1**). One of the biopsy samples from each incision will be fixed in 10% neutral-buffered formalin, routinely processed, and stained with both hematoxylin and eosin, and Masson trichrome (to more clearly identify collagen). Evaluation will be performed by a board-certified dermatopathologist (Steficek), blinded to treatment assignment. Evaluation, scoring and comparison will utilize a standardized, previously published scoring system (**Appendix 7**).[111] The other biopsy sample from each incision will be frozen at -80^{O}F in RNA*later*®.

PILOT DATA:

Data has been collected from the first pilot patient (**Figure 4.3**). On Day 0 a 16cm fusiform incision was marked with a sterile pen. The incision was then made following the drawn lines. The incision was completed until the skin was free and able to fully retract. The original measured width of the incision was 4cm and once the incision was complete, the skin was able to retract to 6cm (**Figure 4.4**). After the ovariohysterectomy was performed, the incision was closed in two layers. First, the linea alba was closed with 2.0 PDS, followed by the skin, which was closed with 3.0 Monocryl. The skin was closed with simple interrupted sutures that were measured and placed 1cm apart. The NPWT system was applied (VAC® Freedom Unit, KCI, San Antonio, TX). Foam and Adaptic® was cut to cover the incision with a 1cm border on each side . The V.A.C. was then turned on and -125mmHg Continuous negative pressure (-125mmHg) was applied to the incision for four days (**Figure 4.5**). On Day 4, Aspen was re-anesthetized and her abdomen was leveled. The prototype tensiometer frame was tested to make sure it was the right size to fit the majority of our future patients. The negative pressure therapy dressing was completely removed. We noticed that there was a significantly sized seroma located at the caudal edge of the wound. Marks were made on either side of the incision, 5mm from the edge of the incision. An 18gauge needle was used to puncture the skin in the same area (**Figure 4.6**). Skin hooks were inserted into the holes created by the 18gauge needle. The skin was then pulled apart slowly until the incision edges were visibly separated (**Figure 4.7**). The force needed to retract the wound edges was measured with a handheld force gauge. The forces needed to separate the skin edges were:

1. Cranial: 0.90lbs
2. Middle: 0.82lbs

3. Caudal: 0.09lbs

We suspect that the presence of the seroma was the reason the force needed to separate the caudal edges of the wound was so minimal compared to the other areas. No biopsies were taken at this time. Photographs were taken at Da y 10 and Day 20 to monitor continued healing of the incision (**Figure 4.8, 4.9**). These images will be used to record the scar scores as previously detailed.

SIGNIFICANCE OF RESEARCH:

We expect that closed incisions under NPWT will demonstrate increased early tensile strength, earlier infiltration of cells associated with wound healing (e.g., fibroblasts), greater levels of collagen, and decreased width and height of scar. Such results would strongly establish the scientific validity of NPWT in closed wounds in all mammalian wound healing (including humans), and would pave the way for a large variety of applications in reconstructive and plastic surgery. A randomized, controlled, veterinary clinical trial for major reconstructive procedures would be undertaken in the MSU VTH.

FURTHER INVESTIGATION:

Further investigations, both human and veterinary, are needed to clarify exactly how NPWT mediates its effects on the various cellular, subcellular and extracellular matrix components of wounds. Further controlled studies comparing NPWT to standard-of-care, and determining optimal length of application and intensity settings need to be conducted. These further studies will allow optimization of its application and an international consensus for use to be reached.

APPENDIX E

Tables and Figures Relevant to Chapter 4

Table 4.1: Form for subjective incision and scar evaluation

Subjective Incision Evaluation and Scar Score

Dog ID:	VAC		CON		Day:
Vascularity	Normal – pale pink				0
	Hyperemic - bright pink				1
	Congested - red				2
	Compromised - purple				3
Height	Flat				0
	<2mm				1
	2-5mm				2
	>5mm				3
Skin Edges MinT Craniad	Edges Sealed				0
	Edges Open				3
	Comment:				
Skin Edges ModT	Edges Sealed				0
	Edges Open				3
	Comment:				
Skin Edges MinT Caudad	Edges Sealed				0
	Edges Open				3
	Comment:				
Periwound Status	Normal tissue				0
	Edema				1
	Erythema				2
	Ulceration				3
	Induration				3
	Discoloration				4

FIGURE 4.1: Diagram of fusiform incision on the ventral abdomen. Marks at 5mm from the wound edge, 2cm & 8cm from each end indicate the placement of tensiometer hooks.

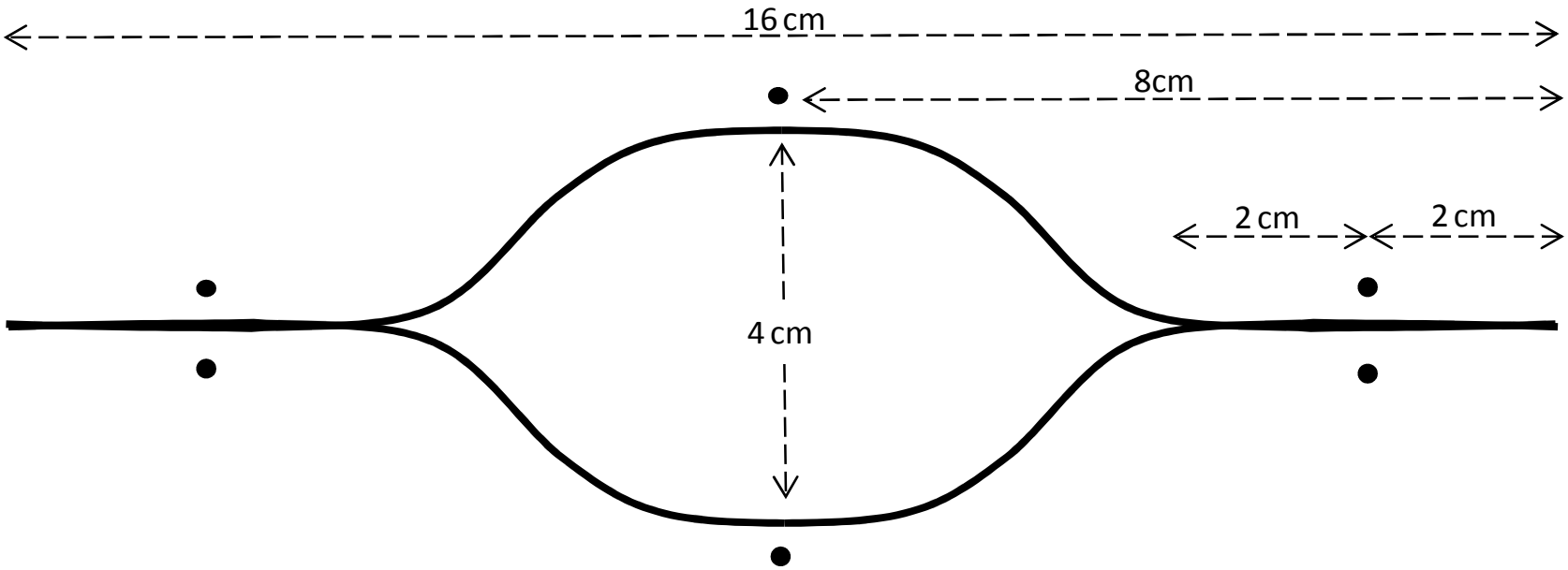


FIGURE 4.2: On Day 4, sutures shown with dotted lines will be removed. The tensiometer will measure force required to just separate wound edges, at the middle mark (under moderate tension, ModT) and at the end marks (under minimal tension, MinT). Following all measurements, remaining sutures will be removed, and 8 mm biopsies will be taken 5 cm from each end, with a disposal dermal biopsy punch.

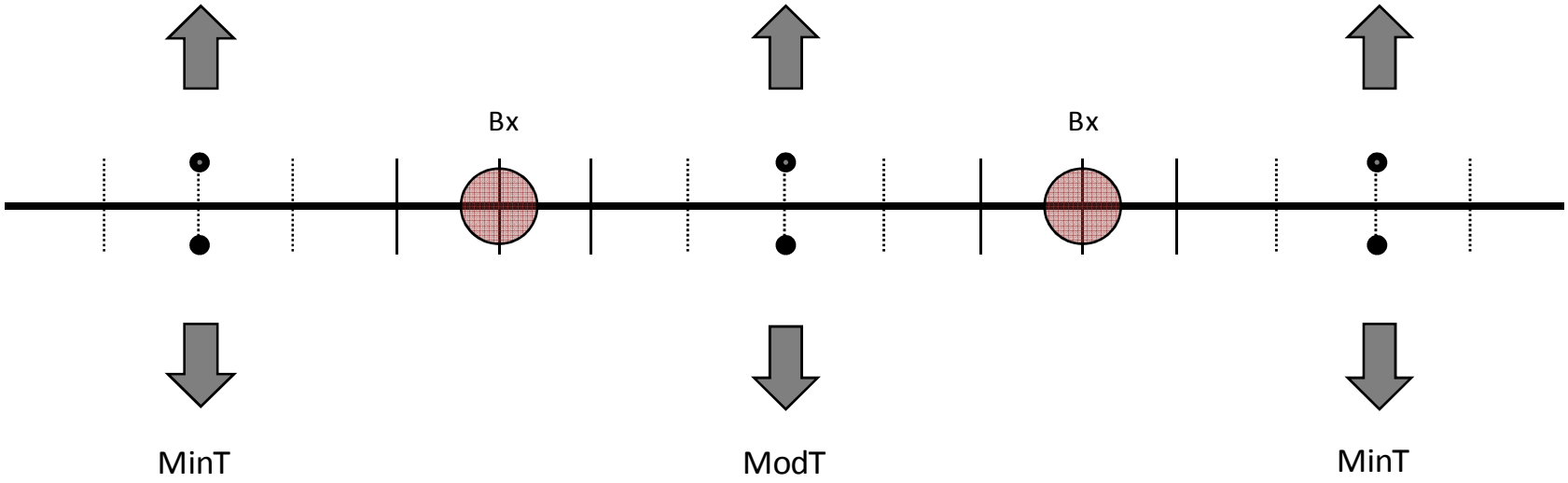
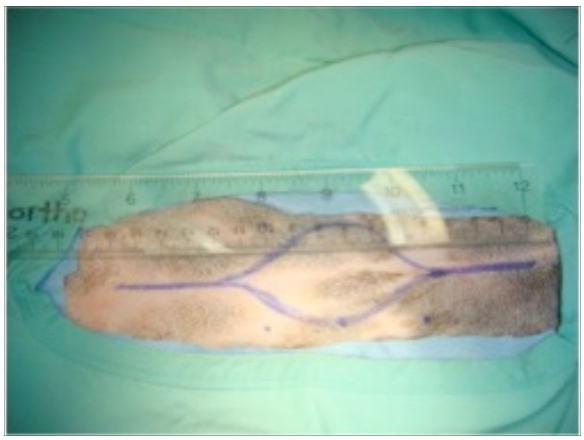


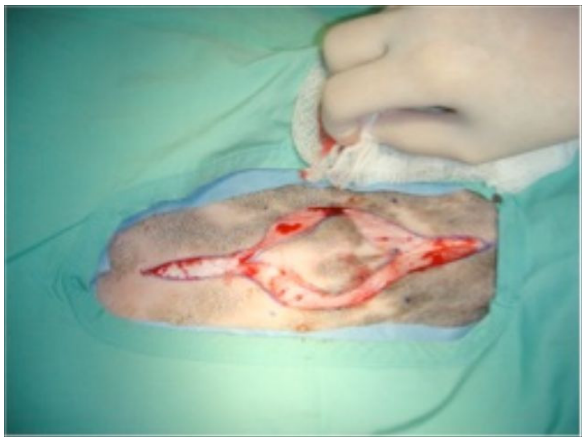
FIGURE 4.3: Our first pilot animal. A 10 month old, German Shorthaired Pointer named Aspen.

FIGURE 4.4: On Day 0 a 16cm fusiform incision was marked with a sterile pen (A). The incision was then made, following the drawn line, with a #15 blade (B). The incision was completed until the skin was free and able to fully retract. The original measured width of the incision was 4cm, which immediately retracted to 6cm (C).

A)



B)



C)

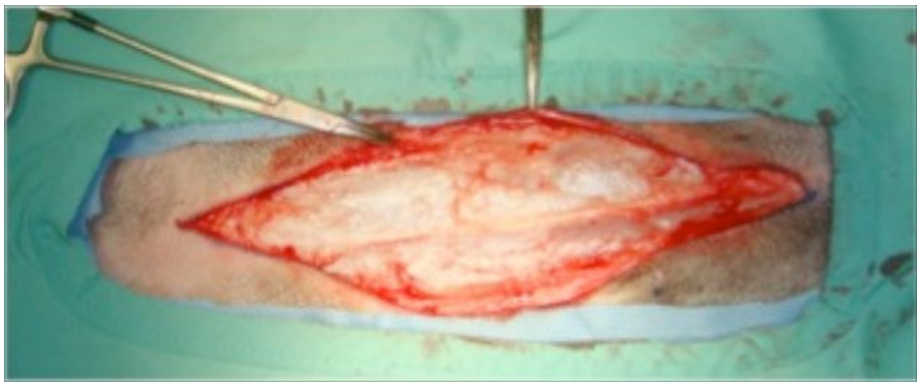
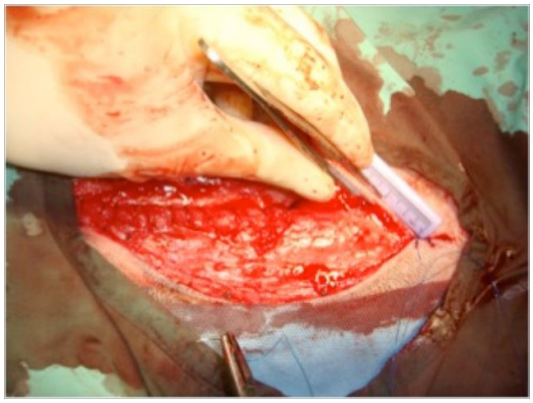
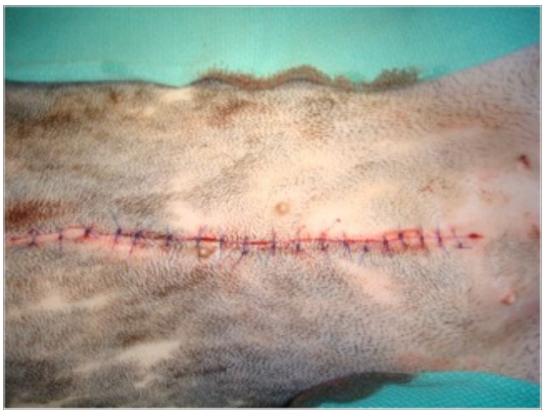


FIGURE 4.5: After the ovariohysterectomy was performed the incision was closed in two layers. First, the linea alba which was closed with 2.0 PDS, followed by the skin which was closed with 3.0 Monocryl. The skin was closed with simple interrupted sutures that were measured and placed 1cm apart (A,B). The NPWT system was applied (VAC® Freedom Unit, KCI, San Antonio, TX). Foam and Adaptic® was cut to cover the incision with a 1cm border on each side (C). The V.A.C. was then turned on and -125mmHg Continuous negative pressure (-125mmHg) was applied to the incision for four days (D).

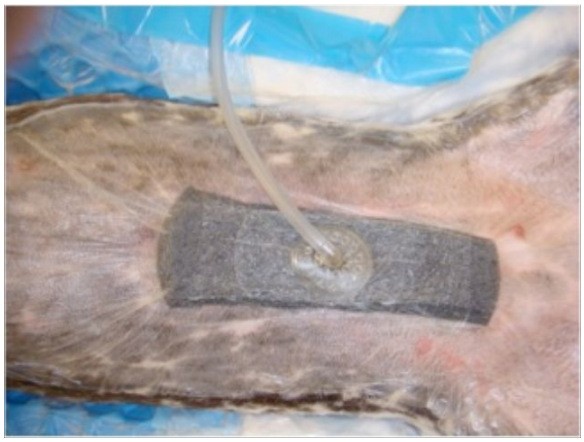
A)



B)



C)



D)

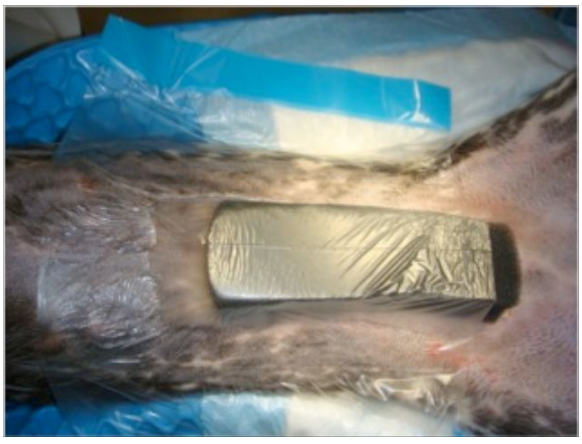
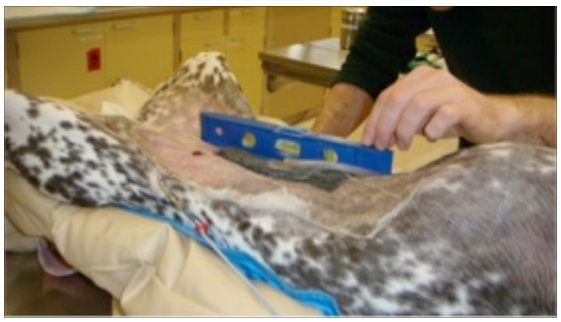


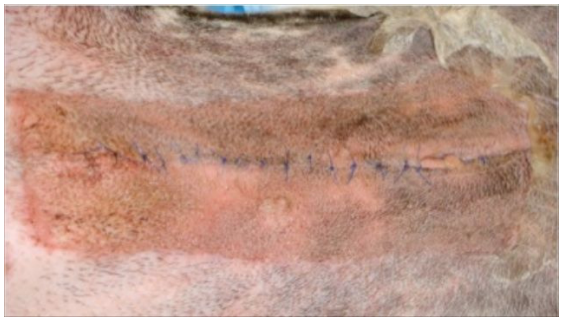
FIGURE 4.6: On Day 4, Aspen was re-anesthetized and her abdomen was leveled (A). The prototype tensiometer frame was tested to make sure it was the right size to fit the majority of our future patients (B). The negative pressure therapy dressing was completely removed (C). Marks were made on either side of the incision, 5mm from the edge of the incision. An 18gauge needle was then used to puncture the skin in the same area (D).

A)



B)

C)



D)

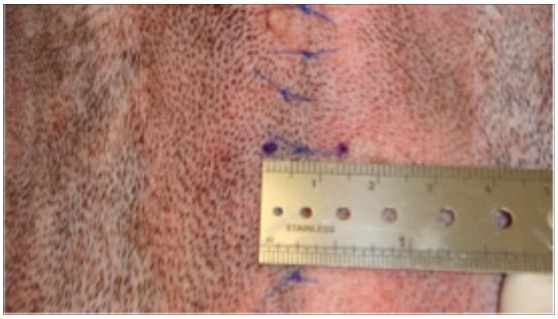
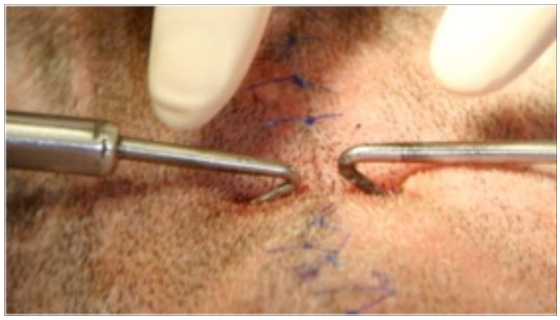
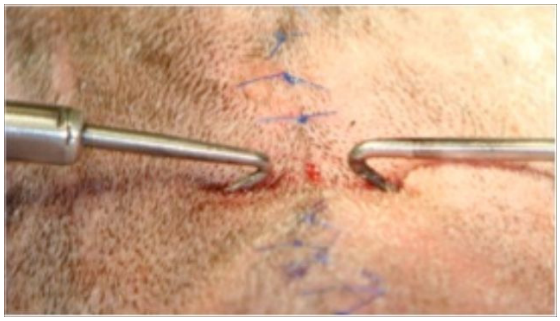


FIGURE 4.7: Homemade skin hooks were inserted into the holes created by the 18gauge needle. The skin was then pulled apart slowly (B) until the incision edges were visibly separated (C). The force needed to retract the wound edges was measured with a handheld force gauge.

A)



B)



C)

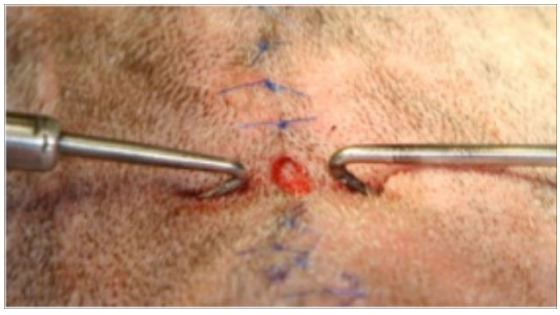


FIGURE 4.8: Photo of the incision 10 days after the original operation. Cranial edge of the wound is to the right, caudal edge to the left.

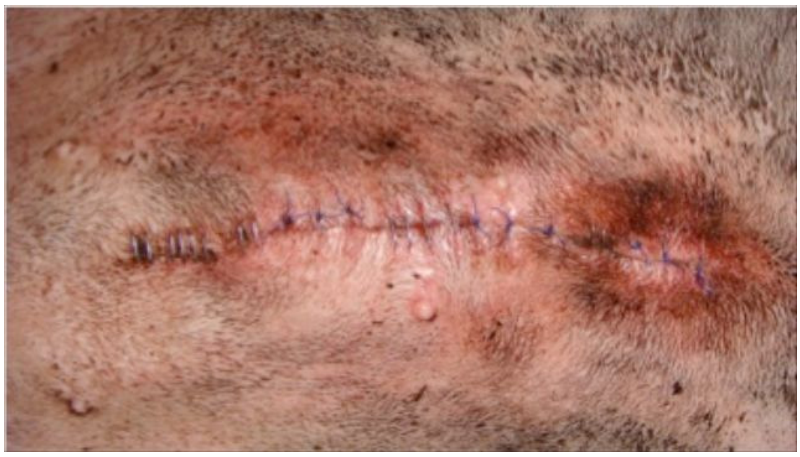
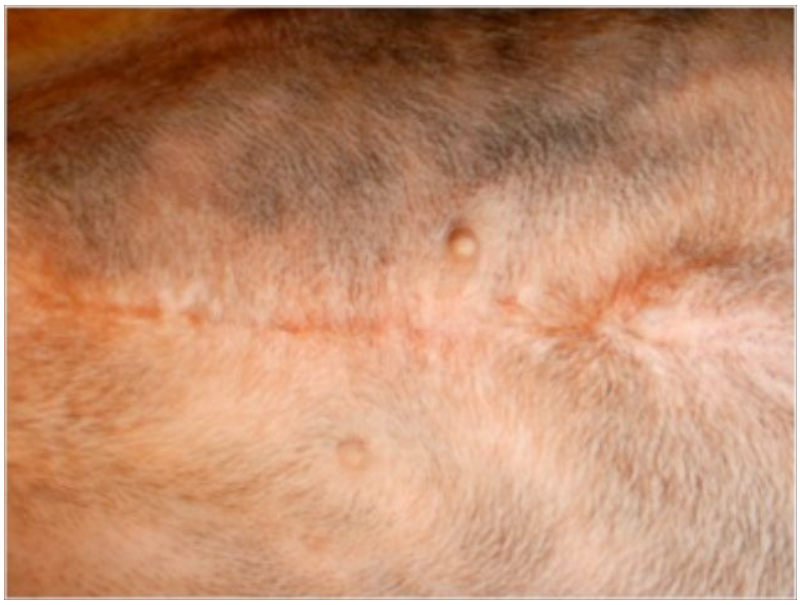


FIGURE 4.9: Photo of the incision 20 days after the original operation. Cranial edge of the wound is to the right, caudal edge to the left.



APPENDIX F

Description of Histologic Acute Inflammation Score

HISTOLOGIC ACUTE INFLAMMATION SCORE (HAIS)

The concentration of neutrophilic cellular infiltration and the degree of edema, hemorrhage, and necrosis will be evaluated and scored: 0=none, 1=minimal, 2=moderate, 3=marked. The criteria used to define the concentration of cellular infiltrates will be as follows: 0=within normal histologic limits, 1=scattered, 2=clustered or nodular, and 3=diffuse. Histologic evaluation of tissue edema will be based primarily on distribution within the sections, with 0=none, 1=focal, 2=localized (regional), and 3=diffuse. The degree or extent of hemorrhage within the tissue sections will be subjectively and comparatively designated as 0=none, 1=mild, 2=moderate, and 3=severe. The necrosis component will be evaluated utilizing the following histopathologic criteria: 0=none, 1=focal, 2=nodular/regional, and 3=diffuse (tracking along fascial planes). These four histologic features will be weighted equally and plotted at each time point to determine any graphical interaction of each composite by group, before being summed to formulate a Histologic Acute Inflammation Score (HAIS; range 0-12).

HISTOLOGIC REPAIR SCORE (HRS)

The following variables will also be evaluated using the same scoring system: fibroblast proliferation, collagen density, and neovascularization. Fibroblast proliferation will be designated, histologically, by pattern and degree of tissue involvement. Scoring criteria will be as follows: 0=none, 1=focal (loose), 2=locally extensive, and 3=effacing normal tissue architecture. Microscopic interpretation of collagen density will be based on intensity and depth of distribution within tissue sections. Scoring values will be defined as 0=none, 1=superficial dermal, 2=superficial to mid dermal, and 3=superficial dermal to subcutaneous. Similar to fibroblast proliferation, the neovascularization component will be scored on histologic pattern and degree of tissue involvement: 0=none, 1=focal (loose), 2=transdermal, and 3=effacing normal tissue architecture. These 3 histologic features will be weighted equally and plotted at each time point to determine any graphical interaction of each composite by group, before being summed to formulate a Histologic Repair Score (HRS; range 0–9).

REFERENCES

REFERENCES

1. Pompeo MQ: The role of "wound burden" in determining the costs associated with wound care. Ostomy Wound Manage 47:65-71, 2001.

2. Frykberg RG, Zgonis T, Armstrong DG, et al: Diabetic foot disorders. A clinical practice guideline (2006 revision). J Foot Ankle Surg 45:S1-66, 2006.

3. Harding KG, Morris HL, Patel GK: Science, medicine and the future: healing chronic wounds. Bmj 324:160-163, 2002.

4. Gordois A, Scuffham P, Shearer A, et al: The health care costs of diabetic peripheral neuropathy in the US. Diabetes Care 26:1790-1795, 2003.

5. Broughton G, 2nd, Janis JE, Attinger CE: A brief history of wound care. Plast Reconstr Surg 117:6S-11S, 2006.

6. Lee B: The Wound Management Manual, in, Vol. New York, The McGraw-Hill Companies, Inc, 2005, p 382.

7. Ovington L: The Evolution of Wound Management. Home Healthc Nurse 20:5, 2002.

8. Medicine NLo: Turning the Pages Online: The Edwin Surgical Papyrus. (http://archive.nlm.nih.gov/proj/ttp/flash/smith/smith.html).

9. Majno G: The healing hand : man and wound in the ancient world. Cambridge, Mass., Harvard University Press, 1975.

10. Celsus AC: De medicina. Cambridge, Mass.,, Harvard university press;, 1935.

11. Nicoli Aldini N, Fini M, Giardino R: From Hippocrates to tissue engineering: surgical strategies in wound treatment. World J Surg 32:2114-2121, 2008.

12. Nutton V: The chronology of Galen's early career. Class Q 23:158-171, 1973.

13. Haller JD: Guy De Chauliac and His Chirurgia Magna. Surgery 55:337-343, 1964.

14. Hamby WB: Ambroise Paré, surgeon of the Renaissance. St. Louis,, W.H. Green, 1967.

15. Forrest RD: Development of wound therapy from the Dark Ages to the present. J R Soc Med 75:268-273, 1982.

16. Shires GT: Evolution of trauma and trauma research. J Trauma 30:S107-115, 1990.

17. Toledo-Pereyra LH, Toledo MM: A critical study of Lister's work on antiseptic surgery. Am J Surg 131:736-744, 1976.

18. Kandela P: Sketches from The Lancet. Antisepsis. Lancet 353:937, 1999.

19. Wangensteen OH, Wangensteen SD, Klinger CF: Surgical cleanliness, hospital salubrity, and surgical statistics, historically considered. Surgery 71:477-493, 1972.

20. Wangensteen OH, Wangensteen SD: Military surgeons and surgery, old and new: an instructive chapter in management of contaminated wounds. Surgery 62:1102-1124, 1967.

21. Greene NM: Anesthesia and the development of surgery (1846-1896). Anesth Analg 58:5-12, 1979.

22. Virchow R: Cellular pathology as based upon physiological and pathological histology. New York,, Dover Publications, 1971.

23. Turnbull PCB: Bacillus, in Baron S (ed): Medical Microbiology (ed 4th), Vol. Galveston (TX), 1996.

24. Morykwas MJ, Argenta LC: Nonsurgical modalities to enhance healing and care of soft tissue wounds. J South Orthop Assoc 6:279-288, 1997.

25. Samson D, Lefevre F, Aronson N: Wound-healing technologies: low-level laser and vacuum-assisted closure. Evid Rep Technol Assess (Summ):1-6, 2004.

26. Swaim SaDK: Wound Management, in Veterinary Clinics of North America, Vol 36. Philadelphia, Saunders, 2006.

27. Tobias KM, Johnston SA: Veterinary surgery : small animal. St. Louis, Mo., Elsevier/Saunders, 2012.

28. Teller P, White TK: The physiology of wound healing: injury through maturation. Surg Clin North Am 89:599-610, 2009.

29. Furie B, Furie BC: Mechanisms of thrombus formation. N Engl J Med 359:938-949, 2008.

30. Kim MH, Liu W, Borjesson DL, et al: Dynamics of neutrophil infiltration during cutaneous wound healing and infection using fluorescence imaging. J Invest Dermatol 128:1812-1820, 2008.

31. Dovi JV, Szpaderska AM, DiPietro LA: Neutrophil function in the healing wound: adding insult to injury? Thromb Haemost 92:275-280, 2004.

32. Toy LW: Matrix metalloproteinases: their function in tissue repair. J Wound Care 14:20-22, 2005.

33. Li J, Zhang YP, Kirsner RS: Angiogenesis in wound repair: angiogenic growth factors and the extracellular matrix. Microsc Res Tech 60:107-114, 2003.

34. Liekens S, De Clercq E, Neyts J: Angiogenesis: regulators and clinical applications. Biochem Pharmacol 61:253-270, 2001.

35. Flegg JA, Byrne HM, Flegg MB, et al: Wound healing angiogenesis: The clinical implications of a simple mathematical model. J Theor Biol, 2012.

36. Jeffcoate WJ, Price P, Harding KG: Wound healing and treatments for people with diabetic foot ulcers. Diabetes Metab Res Rev 20 Suppl 1:S78-89, 2004.

37. Pierce GF, Mustoe TA, Altrock BW, et al: Role of platelet-derived growth factor in wound healing. J Cell Biochem 45:319-326, 1991.

38. Goldman R: Growth factors and chronic wound healing: past, present, and future. Adv Skin Wound Care 17:24-35, 2004.

39. Grinnell F: Fibroblast mechanics in three-dimensional collagen matrices. J Bodyw Mov Ther 12:191-193, 2008.

40. Rhee S, Grinnell F: Fibroblast mechanics in 3D collagen matrices. Adv Drug Deliv Rev 59:1299-1305, 2007.

41. Broughton G, 2nd, Janis JE, Attinger CE: The basic science of wound healing. Plast Reconstr Surg 117:12S-34S, 2006.

42. Ehrlich HP, Krummel TM: Regulation of wound healing from a connective tissue perspective. Wound Repair Regen 4:203-210, 1996.

43. Grinnell F: Fibroblast biology in three-dimensional collagen matrices. Trends Cell Biol 13:264-269, 2003.

44. Morgan PW, Binnington AG, Miller CW, et al: The effect of occlusive and semi-occlusive dressings on the healing of acute full-thickness skin wounds on the forelimbs of dogs. Vet Surg 23:494-502, 1994.

45. Ramsey DT, Pope ER, Wagner-Mann C, et al: Effects of three occlusive dressing materials on healing of full-thickness skin wounds in dogs. Am J Vet Res 56:941-949, 1995.

46. Swaim SF, Gillette RL, Sartin EA, et al: Effects of a hydrolyzed collagen dressing on the healing of open wounds in dogs. Am J Vet Res 61:1574-1578, 2000.

47. Adams AP, Santschi EM, Mellencamp MA: Antibacterial properties of a silver chloride-coated nylon wound dressing. Vet Surg 28:219-225, 1999.

48. Canapp SO, Jr., Farese JP, Schultz GS, et al: The effect of topical tripeptide-copper complex on healing of ischemic open wounds. Vet Surg 32:515-523, 2003.

49. Zumla A, Lulat A: Honey--a remedy rediscovered. J R Soc Med 82:384-385, 1989.

50. Forrest RD: Early history of wound treatment. J R Soc Med 75:198-205, 1982.

51. Tonks AJ, Cooper RA, Jones KP, et al: Honey stimulates inflammatory cytokine production from monocytes. Cytokine 21:242-247, 2003.

52. Anderson I: The properties of hyaluronan and its role in wound healing. Prof Nurse 17:232-235, 2001.

53. Price RD, Myers S, Leigh IM, et al: The role of hyaluronic acid in wound healing: assessment of clinical evidence. Am J Clin Dermatol 6:393-402, 2005.

54. Trabucchi E, Pallotta S, Morini M, et al: Low molecular weight hyaluronic acid prevents oxygen free radical damage to granulation tissue during wound healing. Int J Tissue React 24:65-71, 2002.

55. Marx RE: Platelet-rich plasma (PRP): what is PRP and what is not PRP? Implant Dent 10:225-228, 2001.

56. O'Connell SM, Impeduglia T, Hessler K, et al: Autologous platelet-rich fibrin matrix as cell therapy in the healing of chronic lower-extremity ulcers. Wound Repair Regen 16:749-756, 2008.

57. Hunter JE, Teot L, Horch R, et al: Evidence-based medicine: vacuum-assisted closure in wound care management. Int Wound J 4:256-269, 2007.

58. Meyer W, Schmieden V, Bier AKG: Bier's hyperemic treatment in surgery. Philadelphia and London,, W. B. Saunders company, 1908.

59. Davydov Iu A, Larichev AB, Men'kov KG: [Bacteriologic and cytologic evaluation of vacuum therapy of suppurative wounds]. Vestn Khir Im I I Grek 141:48-52, 1988.

60. Davydov Iu A, Larichev AB, Abramov A, et al: [Concept of clinico-biological control of the wound process in the treatment of suppurative wounds using vacuum therapy]. Vestn Khir Im I I Grek 146:132-136, 1991.

61. Davydov Iu A, Malafeeva EV, Smirnov AP, et al: [Vacuum therapy in the treatment of suppurative lactation mastitis]. Vestn Khir Im I I Grek 137:66-70, 1986.

62. Fleischmann W, Strecker W, Bombelli M, et al: [Vacuum sealing as treatment of soft tissue damage in open fractures]. Unfallchirurg 96:488-492, 1993.

63. Fleischmann W, Lang E, Russ M: [Treatment of infection by vacuum sealing]. Unfallchirurg 100:301-304, 1997.

64. Chariker MJKTT: Effective management of incisional and cutaneous fistulae with closed suction wound drainage. Contemp Surg 34:5, 1989.

65. Argenta LC, Morykwas MJ: Vacuum-assisted closure: a new method for wound control and treatment: clinical experience. Ann Plast Surg 38:563-576; discussion 577, 1997.

66. Morykwas MJ, Argenta LC, Shelton-Brown EI, et al: Vacuum-assisted closure: a new method for wound control and treatment: animal studies and basic foundation. Ann Plast Surg 38:553-562, 1997.

67. Argenta LC, Morykwas MJ, Marks MW, et al: Vacuum-assisted closure: state of clinic art. Plast Reconstr Surg 117:127S-142S, 2006.

68. Karl T, Modic PK, Voss EU: [Indications and results of v.a.C therapy treatments in vascular surgery - state of the art in the treatment of chronic wounds]. Zentralbl Chir 129 Suppl 1:S74-79, 2004.

69. 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 49:55-61; discussion 61, 2002.

70. Avery C, Pereira J, Moody A, et al: Clinical experience with the negative pressure wound dressing. Br J Oral Maxillofac Surg 38:343-345, 2000.

71. Lambert KV, Hayes P, McCarthy M: Vacuum assisted closure: a review of development and current applications. Eur J Vasc Endovasc Surg 29:219-226, 2005.

72. Baharestani MM, Gabriel A: Use of negative pressure wound therapy in the management of infected abdominal wounds containing mesh: an analysis of outcomes. Int Wound J 8:118-125, 2011.

73. Xie X, McGregor M, Dendukuri N: The clinical effectiveness of negative pressure wound therapy: a systematic review. J Wound Care 19:490-495, 2010.

74. Pollak AN, Powell ET, Fang R, et al: Use of negative pressure wound therapy during aeromedical evacuation of patients with combat-related blast injuries. J Surg Orthop Adv 19:44-48, 2010.

75. Dunn R, Hurd T, Chadwick P, et al: Factors associated with positive outcomes in 131 patients treated with gauze-based negative pressure wound therapy. Int J Surg, 2010.
76. Moues CM, Heule F, Legerstee R, et al: Five millennia of wound care products--what is new? A literature review. Ostomy Wound Manage 55:16-18, 20, 22 passim, 2009.

77. Vikatmaa P, Juutilainen V, Kuukasjarvi P, et al: Negative pressure wound therapy: a systematic review on effectiveness and safety. Eur J Vasc Endovasc Surg 36:438-448, 2008.

78. 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 6:185-194, 2005.

79. Moues CM, Heule F, Hovius SE: A review of topical negative pressure therapy in wound healing: sufficient evidence? Am J Surg 201:544-556, 2011.

80. Kumar AR, Grewal NS, Chung TL, et al: Lessons from operation Iraqi freedom: successful subacute reconstruction of complex lower extremity battle injuries. Plast Reconstr Surg 123:218-229, 2009.

81. Leininger BE, Rasmussen TE, Smith DL, et al: Experience with wound VAC and delayed primary closure of contaminated soft tissue injuries in Iraq. J Trauma 61:1207-1211, 2006.

82. Moisidis E, Heath T, Boorer C, et al: A Prospective, Blinded, Randomized, Controlled Clinical Trial of Topical Negative Pressure Use in Skin Grafting. Plast Reconstr Surg 114:917-922, 2004.

83. Vidrine DM, Kaler S, Rosenthal EL: A comparison of negative-pressure dressings versus Bolster and splinting of the radial forearm donor site. Otolaryngol Head Neck Surg 133:403-406, 2005.

84. Cowan KN, Teague L, Sue SC, et al: Vacuum-assisted wound closure of deep sternal infections in high-risk patients after cardiac surgery. Ann Thorac Surg 80:2205-2212, 2005.

85. Lafortune M, Fleming GJ, Wheeler JL, et al: Wound management in a juvenile tiger (Panthera tigris) with vacuum-assisted closure (V.A.C. Therapy). J Zoo Wildl Med 38:341-344, 2007.

86. Ben-Amotz R, Lanz OI, Miller JM, et al: The use of vacuum-assisted closure therapy for the treatment of distal extremity wounds in 15 dogs. Vet Surg 36:684-690, 2007.

87. Adkesson MJ, Travis EK, Weber MA, et al: Vacuum-assisted closure for treatment of a deep shell abscess and osteomyelitis in a tortoise. J Am Vet Med Assoc 231:1249-1254, 2007.

88. Guille AE, Tseng LW, Orsher RJ: Use of vacuum-assisted closure for management of a large skin wound in a cat. J Am Vet Med Assoc 230:1669-1673, 2007.

89. Morykwas MJ, Faler BJ, Pearce DJ, et al: Effects of varying levels of subatmospheric pressure on the rate of granulation tissue formation in experimental wounds in swine. Ann Plast Surg 47:547-551, 2001.

90. Saxena V, Hwang C-W, Huang S, et al: Vacuum-Assisted Closure: Microdeformations of Wounds and Cell Proliferation. Plast Reconstr Surg:1086-1096, 2004.

91. Fabian TS, Kaufman HJ, Lett ED, et al: The evaluation of subatmospheric pressure and hyperbaric oxygen in ischemic full-thickness wound healing. Am Surg 66:1136-1143, 2000.

92. Malmsjo M, Gustafsson L, Lindstedt S, et al: The effects of variable, intermittent, and continuous negative pressure wound therapy, using foam or gauze, on wound contraction, granulation tissue formation, and ingrowth into the wound filler. Eplasty 12:e5, 2012.

93. Jacobs S, Simhaee DA, Marsano A, et al: Efficacy and mechanisms of vacuum-assisted closure (VAC) therapy in promoting wound healing: a rodent model. J Plast Reconstr Aesthet Surg 62:1331-1338, 2009.

94. Weed T, Ratliff C, Drake DB: Quantifying bacterial bioburden during negative pressure wound therapy: does the wound VAC enhance bacterial clearance? Ann Plast Surg 52:276-279; discussion 279-280, 2004.

95. Moues CM, Vos MC, van den Bemd GJ, et al: Bacterial load in relation to vacuum-assisted closure wound therapy: a prospective randomized trial. Wound Repair Regen 12:11-17, 2004.

96. Wackenfors A, Sjogren J, Gustafsson R, et al: Effects of vacuum-assisted closure therapy on inguinal wound edge microvascular blood flow. Wound Repair Regen 12:600-606, 2004.

97. Wackenfors A, Gustafsson R, Sjogren J, et al: Blood flow responses in the peristernal thoracic wall during vacuum-assisted closure therapy. Ann Thorac Surg 79:1724-1730; discussion 1730-1721, 2005.

98. Chen SZ, Li J, Li XY, et al: Effects of vacuum-assisted closure on wound microcirculation: an experimental study. Asian J Surg 28:211-217, 2005.

99. Lindstedt S, Malmsjo M, Ingemansson R: Blood flow changes in normal and ischemic myocardium during topically applied negative pressure. Ann Thorac Surg 84:568-573, 2007.

100. Lindstedt S, Malmsjo M, Sjogren J, et al: Impact of different topical negative pressure levels on myocardial microvascular blood flow. Cardiovasc Revasc Med 9:29-35, 2008.

101. 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 30:85-89, 2006.

102. Timmers MS, Le Cessie S, Banwell P, et al: The effects of varying degrees of pressure delivered by negative-pressure wound therapy on skin perfusion. Ann Plast Surg 55:665-671, 2005.

103. 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 16:460-465, 2008.

104. Chintamani, Singhal V, Singh J, et al: Half versus full vacuum suction drainage after modified radical mastectomy for breast cancer- a prospective randomized clinical trial[ISRCTN24484328]. BMC Cancer 5:11, 2005.

105. Kilpadi DV, Bower CE, Reade CC, et al: Effect of Vacuum Assisted Closure Therapy on early systemic cytokine levels in a swine model. Wound Repair Regen 14:210-215, 2006.

106. 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 66:749-757, 2009.

107. Chen CS, Mrksich M, Huang S, et al: Micropatterned surfaces for control of cell shape, position, and function. Biotechnol Prog 14:356-363, 1998.

108. 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 9:3179-3193, 1998.

109. Scherer LA, Shiver S, Chang M, et al: The vacuum assisted closure device: a method of securing skin grafts and improving graft survival. Arch Surg 137:930-933; discussion 933-934, 2002.

110. 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 244:700-705, 2006.

111. Demaria M, Stanley BJ, Hauptman JG, et al: Effects of negative pressure wound therapy on healing of open wounds in dogs. Vet Surg 40:658-669, 2011.
112. Stannard JP, Volgas DA, Stewart R, et al: Negative pressure wound therapy after severe open fractures: a prospective randomized study. J Orthop Trauma 23:552-557, 2009.

113. Bollero D, Carnino R, Risso D, et al: Acute complex traumas of the lower limbs: a modern reconstructive approach with negative pressure therapy. Wound Repair Regen 15:589-594, 2007.

114. Philbeck TE, Jr., Whittington KT, Millsap MH, et al: The clinical and cost effectiveness of externally applied negative pressure wound therapy in the treatment of wounds in home healthcare Medicare patients. Ostomy Wound Manage 45:41-50, 1999.

115. Gustafsson R, Johnsson P, Algotsson L, et al: Vacuum-assisted closure therapy guided by C-reactive protein level in patients with deep sternal wound infection. J Thorac Cardiovasc Surg 123:895-900, 2002.

116. Fuchs U, Zittermann A, Stuettgen B, et al: Clinical outcome of patients with deep sternal wound infection managed by vacuum-assisted closure compared to conventional therapy with open packing: a retrospective analysis. Ann Thorac Surg 79:526-531, 2005.

117. Sjogren J, Gustafsson R, Nilsson J, et al: Clinical outcome after poststernotomy mediastinitis: vacuum-assisted closure versus conventional treatment. Ann Thorac Surg 79:2049-2055, 2005.

118. Halvorson J, Jinnah R, Kulp B, et al: Use of vacuum-assisted closure in pediatric open fractures with a focus on the rate of infection. Orthopedics 34:e256-260, 2011.
119. DeFranzo AJ, Pitzer K, Molnar JA, et al: Vacuum-assisted closure for defects of the abdominal wall. Plast Reconstr Surg 121:832-839, 2008.

120. Bee TK, Croce MA, Magnotti LJ, et al: Temporary abdominal closure techniques: a prospective randomized trial comparing polyglactin 910 mesh and vacuum-assisted closure. J Trauma 65:337-342; discussion 342-334, 2008.

121. Wild T, Stortecky S, Stremitzer S, et al: [Abdominal dressing -- a new standard in therapy of the open abdomen following secondary peritonitis?]. Zentralbl Chir 131 Suppl 1:S111-114, 2006.

122. Subramonia S, Pankhurst S, Rowlands BJ, et al: Vacuum-assisted closure of postoperative abdominal wounds: a prospective study. World J Surg 33:931-937, 2009.

123. Perez D, Wildi S, Demartines N, et al: Prospective evaluation of vacuum-assisted closure in abdominal compartment syndrome and severe abdominal sepsis. J Am Coll Surg 205:586-592, 2007.

124. Kilpadi DV, Cunningham MR: Evaluation of closed incision management with negative pressure wound therapy (CIM): hematoma/seroma and involvement of the lymphatic system. Wound Repair Regen 19:588-596, 2011.

125. Stannard JP, Robinson JT, Anderson ER, et al: Negative pressure wound therapy to treat hematomas and surgical incisions following high-energy trauma. J Trauma 60:1301-1306, 2006.

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

127. Atkins BZ, Wooten MK, Kistler J, et al: Does negative pressure wound therapy have a role in preventing poststernotomy wound complications? Surg Innov 16:140-146, 2009.

128. Reddix RN, Jr., Tyler HK, Kulp B, et al: Incisional vacuum-assisted wound closure in morbidly obese patients undergoing acetabular fracture surgery. Am J Orthop (Belle Mead NJ) 38:446-449, 2009.

129. Reddix RN, Jr., Leng XI, Woodall J, et al: The effect of incisional negative pressure therapy on wound complications after acetabular fracture surgery. J Surg Orthop Adv 19:91-97, 2010.

130. Karunakar MA, Shah SN, Jerabek S: Body mass index as a predictor of complications after operative treatment of acetabular fractures. J Bone Joint Surg Am 87:1498-1502, 2005.

131. 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 118:390-397; discussion 398-400, 2006.

132. Orgill DP, Bayer LR: Advancing the treatment options of chest wounds with negative pressure wound therapy. Ostomy Wound Manage 51:39S-43S, 2005.

133. Kutschka I, Frauendorfer P, Harringer W: [Vacuum assisted closure therapy improves early postoperative lung function in patients with large sternal wounds]. Zentralbl Chir 129 Suppl 1:S33-34, 2004.

134. Pavletic MM, Pavletic MM: Atlas of small animal wound management and reconstructive surgery (ed 3rd). Ames, Iowa, Wiley-Blackwell, 2010.

135. 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 5:511-529, 2008.

136. Kanakaris NK, Thanasas C, Keramaris N, et al: The efficacy of negative pressure wound therapy in the management of lower extremity trauma: review of clinical evidence. Injury 38 Suppl 5:S9-18, 2007.

137. Parrett BM, Matros E, Pribaz JJ, et al: Lower extremity trauma: trends in the management of soft-tissue reconstruction of open tibia-fibula fractures. Plast Reconstr Surg 117:1315-1322; discussion 1323-1314, 2006.

138. Morykwas MJ, David LR, Schneider AM, et al: Use of subatmospheric pressure to prevent progression of partial-thickness burns in a swine model. J Burn Care Rehabil 20:15-21, 1999.

139. Morykwas MJ, Kennedy A, Argenta JP, et al: Use of subatmospheric pressure to prevent doxorubicin extravasation ulcers in a swine model. J Surg Oncol 72:14-17, 1999.

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

141. Peinemann F, Sauerland S: Negative-pressure wound therapy: systematic review of randomized controlled trials. Dtsch Arztebl Int 108:381-389, 2011.

142. Glass GE, Nanchahal J: The methodology of negative pressure wound therapy: Separating fact from fiction. J Plast Reconstr Aesthet Surg, 2012.

143. Grellner D: Pers. comm., in, Vol Kinetic Concepts Inc., San Antonio, TX, 2012.

144. Genecov DG, Schneider AM, Morykwas MJ, et al: A controlled subatmospheric pressure dressing increases the rate of skin graft donor site reepithelialization. Ann Plast Surg 40:219-225, 1998.

145. Moisidis E, Heath T, Boorer C, et al: A prospective, blinded, randomized, controlled clinical trial of topical negative pressure use in skin grafting. Plast Reconstr Surg 114:917-922, 2004.

146. Schneider AM, Morykwas MJ, Argenta LC: A new and reliable method of securing skin grafts to the difficult recipient bed. Plast Reconstr Surg 102:1195-1198, 1998.

147. Paul JC: Vacuum assisted closure therapy: a must in plastic surgery. Plast Surg Nurs 25:61-65, 2005.

148. Uygur F, Duman H, Ulkur E, et al: The role of the vacuum-assisted closure therapy in the salvage of venous congestion of the free flap: case report. Int Wound J 5:50-53, 2008.

149. Argenta PA, Rahaman J, Gretz HF, 3rd, et al: Vacuum-assisted closure in the treatment of complex gynecologic wound failures. Obstet Gynecol 99:497-501, 2002.

150. de Vooght A, Feruzi G, Detry R, et al: Vacuum-assisted closure for abdominal wound dehiscence with prosthesis exposure in hernia surgery. Plast Reconstr Surg 112:1188-1189, 2003.

151. Fife CE, Otto G, Walker D, et al: Healing dehisced surgical wounds with negative pressure wound therapy. Ostomy Wound Manage 50:28-31, 2004.

152. Fackler ML: Vacuum-assisted closure of the abdomen. J Am Coll Surg 206:400, 2008.

153. Stone PA, Hass SM, Flaherty SK, et al: Vacuum-assisted fascial closure for patients with abdominal trauma. J Trauma 57:1082-1086, 2004.

154. Schlatterer D, Hirshorn K: Negative pressure wound therapy with reticulated open cell foam-adjunctive treatment in the management of traumatic wounds of the leg: a review of the literature. J Orthop Trauma 22:S152-160, 2008.

155. Pollak AN: Use of negative pressure wound therapy with reticulated open cell foam for lower extremity trauma. J Orthop Trauma 22:S142-145, 2008.

156. Terrazas SG: Adjuvant dressing for negative pressure wound therapy in burns. Ostomy Wound Manage 52:16, 18, 2006.

157. Nugent N, Lannon D, O'Donnell M: Vacuum-assisted closure - a management option for the burns patient with exposed bone. Burns 31:390-393, 2005.

158. Lalliss SJ, Branstetter JG, Murray CK, et al: Infection rates in U.S. military personnel using vacuum-assisted closure. Plast Reconstr Surg 120:574-575; author reply 575-576, 2007.

159. Powell ETt: The role of negative pressure wound therapy with reticulated open cell foam in the treatment of war wounds. J Orthop Trauma 22:S138-141, 2008.

160. Geiger S, McCormick F, Chou R, et al: War wounds: lessons learned from Operation Iraqi Freedom. Plast Reconstr Surg 122:146-153, 2008.

161. Kumar AR, Grewal NS, Chung TL, et al: Lessons from the modern battlefield: successful upper extremity injury reconstruction in the subacute period. J Trauma 67:752-757, 2009.

162. Marsh DJ, Abu-Sitta G, Patel H: The role of vacuum-assisted wound closure in blast injury. Plast Reconstr Surg 119:1978-1979, 2007.

163. Hinck D, Franke A, Gatzka F: Use of vacuum-assisted closure negative pressure wound therapy in combat-related injuries--literature review. Mil Med 175:173-181, 2010.

164. Apelqvist J, Armstrong DG, Lavery LA, et al: Resource utilization and economic costs of care based on a randomized trial of vacuum-assisted closure therapy in the treatment of diabetic foot wounds. Am J Surg, 2008.

165. Niezgoda JA: The economic value of negative pressure wound therapy. Ostomy Wound Manage 51:44S-47S, 2005.

166. Clark T: MSU CVM Wounds, in Stanley DB (ed): (ed E-mail communication), Vol, 2012.

167. Malmsjo M, Ingemansson R, Martin R, et al: Wound edge microvascular blood flow: effects of negative pressure wound therapy using gauze or polyurethane foam. Ann Plast Surg 63:676-681, 2009.

168. Malmsjo M, Ingemansson R, Martin R, et al: 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 17:200-205, 2009.

169. Harrison TM, Stanley BJ, Sikarskie JG, et al: Surgical amputation of a digit and vacuum-assisted closure (V.A.C.) management in a case of osteomyelitis and wound care in an Eastern Black Rhinoceros (*Diceros bicornis michaeli*). Journal of Zoo and Wildlife Medicine 42:317-321, 2011.

170. Owen L, Hotston-Moore A, Holt P: Vacuum-assisted wound closure following urine-induced skin and thigh muscle necrosis in a cat. Vet Comp Orthop Traumatol 22:417-421, 2009.

171. Gemeinhardt KD, Molnar JA: Vacuum-assisted closure for management of a traumatic neck wound in a horse. Equine Veterinary Education 17:27-33, 2005.

172. Stanley BJ, Pitt, K.A, et. al.: Effects of Negative Pressure Wound Therapy on Healing of Full-Thickness Skin Grafts in Dogs. Veterinary Surgery, 2012- In press.

173. Medical T: Venturi (TM) Negative Pressure Wound Therapy System: Clinical Guidelines Manual, in, Vol.

174. Kinetic Concepts I: V.A.C. Therapy Clinical Guidelines: A reference source for clinicians, in, Vol, 2007.

175. Kinetic Concepts I: V.A.C. Therapy(TM) Clinical Guidelines: A reference source for clinicians, in, Vol, 2005.

176. Nephew S: EZCare Negative Pressure Wound Therapy: User Guide, in, Vol, 2007.
177. UNIVERSITY MS, HOSPITAL VT: SMALL ANIMAL ANESTHESIA ROTATION HANDOUT. (http://old.cvm.msu.edu/services/aneth/docs/Anesrot.htm), AUGUST 2011.

178. Plumb DC: Plumb's veterinary drug handbook (ed 7th). Stockholm, Wis. Ames, Iowa, PharmaVet ; Distributed by Wiley, 2011.

179. Chang KP, Tsai CC, Lin TM, et al: An alternative dressing for skin graft immobilization: negative pressure dressing. Burns 27:839-842, 2001.

180. Apelqvist J, Armstrong DG, Lavery LA, et al: Resource utilization and economic costs of care based on a randomized trial of vacuum-assisted closure therapy in the treatment of diabetic foot wounds. Am J Surg 195:782-788, 2008.

181. Baharestani MM: Negative pressure wound therapy: an examination of cost-effectiveness. Ostomy Wound Manage 50:29S-33S, 2004.

182. Mokhtari A, Sjogren J, Nilsson J, et al: The cost of vacuum-assisted closure therapy in treatment of deep sternal wound infection. Scand Cardiovasc J 42:85-89, 2008.

183. Slatter DH: Textbook of small animal surgery. Philadelphia, W.B. Saunders, 1985.

184. Waris T, Rechardt L, Kyosola K: Reinnervation of human skin grafts: a histochemical study. Plast Reconstr Surg 72:439-447, 1983.

185. DeCarbo WT, Hyer CF: Negative-pressure wound therapy applied to high-risk surgical incisions. J Foot Ankle Surg 49:299-300, 2010.

186. Dunn R, Hurd T, Chadwick P, et al: Factors associated with positive outcomes in 131 patients treated with gauze-based negative pressure wound therapy. Int J Surg 9:258-262, 2011.

187. Petzina R, Hoffmann J, Navasardyan A, et al: Negative pressure wound therapy for post-sternotomy mediastinitis reduces mortality rate and sternal re-infection rate compared to conventional treatment. Eur J Cardiothorac Surg 38:110-113, 2010.

188. Thompson JT, Marks MW: Negative pressure wound therapy. Clin Plast Surg 34:673-684, 2007.

189. Mendez-Eastman S: Guidelines for using negative pressure wound therapy. Adv Skin Wound Care 14:314-322; quiz 324-315, 2001.

190. Molnar JA: The science behind negative pressure wound therapy. Ostomy Wound Manage 50:2-5, 2004.

191. Scherer SS, Pietramaggiori G, Mathews JC, et al: The mechanism of action of the vacuum-assisted closure device. Plast Reconstr Surg 122:786-797, 2008.

192. Weston C: The science behind topical negative pressure therapy. Acta Chir Belg 110:19-27, 2010.

193. Malli S: Keep a close eye on vacuum-assisted wound closure. Nursing 35:25, 2005.

194. Goldstein JA, Iorio ML, Brown B, et al: The use of negative pressure wound therapy for random local flaps at the ankle region. J Foot Ankle Surg 49:513-516, 2010.

195. Tang AT, Okri SK, Haw MP: Vacuum-assisted closure to treat deep sternal wound infection following cardiac surgery. J Wound Care 9:229-230, 2000.

196. Yuan-Innes MJ, Temple CL, Lacey MS: Vacuum-assisted wound closure: a new approach to spinal wounds with exposed hardware. Spine (Phila Pa 1976) 26:E30-33, 2001.

197. Blackburn JH, 2nd, Boemi L, Hall WW, et al: Negative-pressure dressings as a bolster for skin grafts. Ann Plast Surg 40:453-457, 1998.

198. DeFranzo AJ, Marks MW, Argenta LC, et al: Vacuum-assisted closure for the treatment of degloving injuries. Plast Reconstr Surg 104:2145-2148, 1999.

199. Gregor S, Maegele M, Sauerland S, et al: Negative pressure wound therapy: a vacuum of evidence? Arch Surg 143:189-196, 2008.

200. Mendonca DA, Papini R, Price PE: Negative-pressure wound therapy: a snapshot of the evidence. Int Wound J 3:261-271, 2006.

201. Landau AG, Hudson DA, Adams K, et al: Full-thickness skin grafts: maximizing graft take using negative pressure dressings to prepare the graft bed. Ann Plast Surg 60:661-666, 2008.

202. Mullally C, Carey K, Seshadri R: Use of a nanocrystalline silver dressing and vacuum-assisted closure in a severely burned dog. J Vet Emerg Crit Care (San Antonio) 20:456-463, 2010.

203. Saxena V, Hwang CW, Huang S, et al: Vacuum-assisted closure: microdeformations of wounds and cell proliferation. Plast Reconstr Surg 114:1086-1096; discussion 1097-1088, 2004.

204. Fisher A, Brady B: Vacuum assisted wound closure therapy. Issues Emerg Health Technol:1-6, 2003.

205. Hauser J, Lehnhardt M, Daigeler A, et al: Photoplanimetric evaluation and impedance measurement of split-thickness skin grafts: a new model for objective wound-healing assessment in clinical trials. Skin Res Technol 15:168-171, 2009.

206. Dieu T, Leung M, Leong J, et al: Too much vacuum-assisted closure. ANZ J Surg 73:1057-1060, 2003.

207. Greer SE: Whither subatmospheric pressure dressing? Ann Plast Surg 45:332-334; discussion 335-336, 2000.

208. Kairinos N, Solomons M, Hudson DA: The paradox of negative pressure wound therapy--in vitro studies. J Plast Reconstr Aesthet Surg 63:174-179, 2010.

209. Miller MS, Lowery CA: Negative pressure wound therapy: "a rose by any other name". Ostomy Wound Manage 51:44-46, 48-49, 2005.

210. Mirsaidi N: Negative pressure wound therapy: Use with care. Nursing 40:64, 66, 2010.

211. Morris GS, Brueilly KE, Hanzelka H: Negative pressure wound therapy achieved by vacuum-assisted closure: Evaluating the assumptions. Ostomy Wound Manage 53:52-57, 2007.

212. Preston G: An overview of topical negative pressure therapy in wound care. Nurs Stand 23:62-64, 66, 68, 2008.

213. Ubbink DT, Westerbos SJ, Nelson EA, et al: A systematic review of topical negative pressure therapy for acute and chronic wounds. Br J Surg 95:685-692, 2008.

214. Pavletic MM: The Integument, in Slatter DH (ed): Textbook of Small Animal Surgery (ed 3rd), Vol 1. Philadelphia, Saunders, 2003, pp 250-259.

215. Wilkes RP, Kilpadi DV, Zhao Y, et al: Closed Incision Management With Negative Pressure Wound Therapy (CIM): Biomechanics. Surg Innov, 2011.