REVIEW ARTICLES

Negative Pressure Wound Therapy: Basic Science Review

Authors

[1]Dominic Massary III MD

[2]Michael Morykwas PhD

Affiliations

[1]Resident, Department of Plastic and Reconstructive Surgery, Wake Forest School of Medicine

[2]Department of Plastic and Reconstructive Surgery, Wake Forest School of Medicine

***Corresponding author:**

Dominic Massary III MD

Department of Plastic and Reconstructive Surgery, Wake Forest School of Medicine

Email: mmorykwa@wakehealth.edu

Abstract

Since the initial publication in 1997 of the basic research behind Negative Pressure Wound Therapy and the additional review in 2006, much additional research has been conducted. This short review highlights some of this additional basic research into the mechanisms and physiological responses to the controlled applicaiton of sub-atmospheric pressure for wound care.

Introduction

Since our initial clinical study published in 1997,[1] negative pressure wound therapy has gained wide acceptance. KCI states that their device has been used on millions of patients worldwide, and a quick pubmed search for, "negative pressure wound therapy," generates thousands of results.

Nearly 13 years ago we published a review article on the state of basic research[2] focusing on blood flow, granulation tissue, bacterial clearance, and fluid analysis. This article highlighted novel applications of negative pressure, such as burn wounds and flap salvage, along with discussing the two most accepted mechanisms of action, fluid removal and mechanical deformation. This paper also highlighted inconsistencies within the literature regarding the effect on bacterial clearance.

This review will serve as an update to our 2006 article focusing on studies completed since then, with a closer look at bacterial clearance, blood flow, and neovascularization. Insight has been gained into the effects of negative pressure on different bacterial virulence factors, gene and protein regulation, and blood flow to the wound. This, along with development into new foams and irrigation techniques and formulations, provides critical insight into the mechanisms of negative pressure wound therapy. With greater understanding of these underlying mechanisms we hope that clinicians can take a more individualized approach and tailor treatment to optimize wound healing for each patient's specific needs.

Bacterial Clearance

Since the initial animal model describing a reduction in bacterial burden with negative pressure in pigs inoculated with Staphylococcus aureus and Staphylococcus epidermidis in 1997,[3] much has been published on the relationship of bacterial burden and negative pressure wound therapy. Some studies have shown no effect or an increase in bacterial burden with negative pressure, [4, 5] while others have shown a decrease in bacterial burden.[6, 7] More recently it has become clear that the effect of negative pressure on bacterial burden has become more nuanced.

An *in vitro* study of Pseudomonas aeruginosa and Staphylococcus aureus biofilms demonstrated a reduction in biofilm thickness and mass with negative pressure along with a synergistic effect and further reduction in mass and thickness with negative pressure and irrigation.[8] In another *in vitro* study the importance of selecting an appropriate foam dressing for reducing bacterial burden of Staphylococcus aureus was highlighted. This showed superiority with irrigating with octenidine or application of a silver based

polyurethane foam when compared to a non-antimicrobial polyurethane foam.[9]

An animal study with mice used gold nanoparticles and CT imaging to track Escherichia coli quantitatively. This study showed a reduction in bacterial load by 5% with negative pressure alone, and up to 45% with negative pressure and irrigation.[10] In another animal study using rabbits, it was shown that negative pressure not only decreased the bacterial load of Pseudomonas aeruginosa compared to atmospheric pressure, but also that negative pressure reduced virulence factors, quantity of biofilm, and inhibited motility of Pseudomonas aeruginosa.[11] And in a separate animal study using rabbits it was demonstrated that negative pressure decreased virulence factors and depth of invasion of Staphylococcus aureus compared to atmospheric pressure.[12]

A study involving patients with contaminated wounds, defined as either chronic or acute wounds requiring at least 4 operative interventions, determined that negative pressure along with irrigation reduced bacterial loads independent of wound site or wound type. These patients were not, however, compared to a control group.[13]

These studies have highlighted that the effect of negative pressure on bacterial burden is species dependent and can be augmented with different infusions or foam materials. These studies have highlighted possibilities into conflicting studies on bacterial clearance in the past.[14-16] Further research needs to be done to provide insight into ideal antimicrobial foams and infusions for specific patient scenarios.

Blood Flow

Blood flow with negative pressure dressings has been somewhat controversial in the literature, with conflicting studies and differing methods to assess blood flow and perfusion.

In an animal study using swine, researchers measured coronary blood flow using electromagnetic flow meter probes before and after inducing ischemia by LAD occlusion for 20 minutes and treating with topical negative pressure. This study showed an increase in coronary blood flow in both normal and ischemic myocardium when topical negative pressure was applied.[17] Another swine study treated full thickness soft tissue wounds with negative pressure and assessed the blood flow at the wound edges. Blood flow was measured using thermodiffusion, transcutaneous laser Doppler, and Invasive laser Doppler. When using thermodiffusion and invasive laser Doppler, it was shown that at the wound edges blood flow decreased but moving further away from the edge blood flow increased. However, when using transcutaneous laser Doppler it was

shown that there was an increase in blood flow around the wound no matter the proximity.[18]

In healthy volunteers the application of a cutaneous negative pressure dressing showed via echo-color-Doppler increased flowmetery of the periumbilical artery perforators after the dressing was removed.[19] Another study with healthy volunteers showed increased blood flow, oxygen saturation, and relative hemoglobin content under intermittent negative pressure, however, the authors noted patient discomfort with the intermittent application of negative pressure, possibly limiting the utility.[20]

In patients with spinal cord injury, intermittent negative pressure was applied to the lower leg and an increase in pedal blood flow was observed using laser Doppler and ultrasound Doppler.[21] While a similar study, by the same group, they showed improvement in pedal blood flow with negative pressure application in patients with peripheral vascular disease.[22]

It has also been noted in the literature that the increase in blood flow when measuring using laser Doppler could be the result of compression forces decreasing the diameter of the blood vessels increasing the flow while decreasing perfusion, especially with circumferential application of the dressing.[23] This study using radioisotope perfusion imaging and transcutaneous partial pressures on healthy patients showed a decrease in perfusion with negative pressure.[23] Another study on patients with diabetic foot ulcers showed decreased transcutaneous partial oxygen pressures with negative pressure application.[24]

The differences in outcomes of these studies seem to be related to the method of measuring blood flow and tissue perfusion. Further investigation into the interpretation of laser Doppler and ultrasound Doppler are needed to fully understand the effect of negative pressure on blood flow. A message to be taken when looking at this data is that it is universally accepted that caution should be taken when applying negative pressure circumferentially or with pressures in excess of -125mmHg, as this has the potential for decreased blood flow to the wound bed or distal extremity.

Granulation and neovascularization

Much of the histologic study of negative pressure has been done using animal models. An animal study using dogs showed negative pressure treated wounds were significantly faster to close, with greater neovascularization, and more organized granulation tissue when compared to foam treated wounds alone.[25] Negative pressure wound therapy has also been shown to increase blood vessel density around prosthetics in hairless rats.[26] In a rabbit model with exposed cranial bone, negative pressure therapy increase wound healing, vessel density, increased hydroxyproline content, and

generated ordered collagen arrangement.[27] While the observation of increased vascularity and granulation tissue with negative pressure has been universal, the underlying mechanism still remains unclear.

One study has shown mobilization of fibroblasts with negative pressure is a potential mechanism for improved wound healing. In mice that received tail vein injections of PKH-26 labeled fibroblasts they showed that when negative pressure is applied fibroblast mobilization is increased. They also demonstrate that when fibroblast mobilization is inhibited and negative pressure is applied the mice show decreases in mRNA and protein expression, blood vessel density, and proliferating cells similar to wounds treated without negative pressure.[28]

Negative pressure has also been shown to alter expression of mRNA and transcription of proteins involved in angiogenesis and inflammation. Multiple studies have shown an increase in TGF-B1, VEGF, TIMP1, GFRA2, C1QBP, RAB35, and SYNJ1 expression, and downregulation of IL1B, IL6, iNOS, TNFa, MMP1, MMP8, and MMP9,[28-34] all thought to be mechanisms of increased wound healing.

It has also been shown that the addition of bone marrow mesenchymal stem cells *in vitro* with negative pressure increased proliferation and differentiation into the angiogenic related cells. *In vivo*, rat wounds treated with BMSCs combined with NPWT exhibited better viability and enhanced angiogenesis. There was also enhanced expression of NG2, VEGF, CD31, and a-SMA when compared to negative pressure alone.[35]

Another observation is that keratinocytes have greater movement under negative pressure. An *in vitro* study was designed showing negative pressure down regulates E-cadherin through induction of p120-catenin, ultimately promoting keratinocyte movement. This effect is thought to be induced by mechanical forces acting on the keratinocytes at the epithelial adherens junction.[36] The mechanical stress at the adherens junction causes phosphorylation of p120-catenin under negative pressure leading to endocytosis of E-cadherin. The loss of E-cadherin decreases cell-cell adhesion and allows for cell migration.

In summary, much research has been done on the basic science of negative pressure wound therapy. The focus on basic science has given crucial insight into treatment optimization. Augmentation of treatment with irrigation solutions, foam formulations, timing and frequency of negative pressure, or supplementation with progenitor cells, have all given clinicians more options for a more individualized approach in the treatment of non-healing wounds.

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