

The influence of vacuum assisted closure therapy on bacterial growth and biofilm development in the wounds

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UNIVERSITY OF ZAGREB
SCHOOL OF MEDICINE

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**The influence of vacuum assisted closure therapy
on bacterial growth and biofilm development in
the wounds**

Graduate thesis



Zagreb, 2021.

This graduate thesis was made at the Department for Plastic and Reconstructive Surgery, at University Hospital Center, KBC Rebro in Zagreb, mentored by Assistant professor Krešimir Bulić, and was submitted for evaluation in the academic year 2020/2021.

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Summary

Vacuum assisted closure therapy was developed for the needs of plastic surgery in the 90s. Primarily used for the treatment of patients with large chronic wounds, the usage nowadays extends to area of medicine and veterinary, for all types of large, chronic and infected wounds, where increased drainage of the exudate and help with wound closure is needed. Vacuum assisted closure therapy uses sub-atmospheric pressure between 50 and 200 mmHg which facilitates drainage of fluids, reduces swelling, mechanically removes bacteria and increases blood flow. Precaution is needed with poorly debrided wounds, with irradiated tissue, with fragile blood vessels and with patients on anticoagulation therapy.

The foam with 400 to 600 micrometer pores, covers the wound area completely, over which protective seal is placed. Tubing connects the seal with the vacuum pump, on which continuous, variable or intermittent mode of action and level of negative pressure are set.

Since the early nineties numerous research articles were written on the topic of negative pressure and vacuum therapy and they mostly agree, that utilisation of negative pressure improves rate of granulation tissue formation, decreases time of healing and assists with infectious process in the wound. In-vitro and in-vivo research shows good clinical results. Additionally, gene testing and microbiological results are encouraging.

Key words: Vacuum assisted closure therapy, negative pressure, chronic wound treatment, biofilms

Sažetak

Terapija kroničnih rana uz pomoć vakuuma razvijena je za potrebe plastične kirurgije u početku 90-ih godina. Primarno korišten za liječenje bolesnika s velikim kroničnim ranama, danas se koristi na području medicine i veterine, za sve vrste velikih, kroničnih i zaraženih rana, gdje je potrebna povećana drenaža eksudata i pomoć pri zatvaranju rana. Terapija negativnim tlakom koristi subatmosferski tlak između 50 i 200 mmHg što olakšava drenažu tekućina, smanjuje oticanje, mehanički uklanja bakterije i povećava protok krvi. Potreban je oprez sa loše očišćenim ranama, s ozračenim tkivom, krhkim krvnim žilama i s pacijentima na antikoagulacijskoj terapiji.

Pjena s porama od 400 do 600 mikrometara u potpunosti pokriva područje rane preko koje se postavlja zaštitna folija. Cijev spaja zaštitnu foliju s vakuumskom pumpom, na kojoj se kontroliraju kontinuirani, promjenjivi ili isprekidani način djelovanja i razina negativnog tlaka. Od početka devedesetih na temu negativnog tlaka i vakuumske terapije napisani su brojni istraživački članci, koji se uglavnom slažu da korištenje negativnog tlaka poboljšava brzinu stvaranja granulacijskog tkiva, smanjuje vrijeme zacjeljivanja i pomaže kod zaraznog procesa u rani. In-vitro i in-vivo istraživanja pokazuju dobre kliničke rezultate. Također ohrabruju rezultati genetskog ispitivanja i mikrobiološki rezultati.

Ključne riječi: Terapija negativnim tlakom, negativni tlak, liječenje kronične rane, biofilmi

1. Introduction

Vacuum assisted closure is a non-invasive wound management therapy used most often in difficult-to-manage wound care (1). It was developed by Dr. Argenta and Dr. Morykows for the field of plastic surgery in the early 90s in the USA, and it was first used in Europe in Germany during the 90s (2).

It is known under pseudonyms:

- VAC (vacuum assisted closure),
- TNP (topical negative pressure),
- SPD (sub-atmospheric pressure),
- VST (vacuum sealing technique) and
- SSS (sealed surface wound suction) (3).

The system can create a sub-atmospheric pressure optimal for wound healing between 50 mmHg and 200 mmHg, however, studies have shown that values between -50 mmHg and -125 mmHg show optimal results. Depending on the type of wound and healing phase, the pressure is either continuous or uses an alternating pressure cycle (4)(5).

By decreasing the pressure over the area of the wound, the fluid is gently drained, which reduces swelling, helps clean the wound and removes bacteria (6). In addition it keeps the wound moist and warm, draws edges closer together and increases blood flow (5).

Studies comparing utilisation of negative pressure therapy in the treatment of chronic wounds to those treatments using conventional dressing showed drastic improvement in the speed of healing as well as decreased sensation of pain in the majority of cases.

A study of wound healing in patients with diabetic foot ulcer compared the amount of bleeding or infection, between groups treated with negative pressure and treated with conventional dressings. Mean healing time as well as total or almost total granulation tissue coverage improved significantly, with 22.52 to 3.85 days ($P < 0.0001$) and 23.33 to 32.15

days ($P < 0.0001$) days respectively. Rate of granulation tissue was significantly better with negative pressure therapy with $2.91 \text{ cm}^2/\text{day}$ versus $2.16 \text{ cm}^2/\text{day}$ ($P = 0.0306$). The intensity of the pain on week 3 was as well significantly decreased in patients using vacuum assisted closure (7). Furthermore, retrospective study on 20 patients with traumatic infected wounds showed 29% mean reduction of wound area with continuous mode of usage of negative pressure wound therapy. Additionally, no complications directly caused by negative pressure therapy were observed (8).

An animal study published in the Annals of Plastic Surgery confirmed the findings in a series of basic animal studies by showing that vacuum assisted closure significantly increased rates of granulation tissue formation ($p \leq 0.05$) both with continuous ($63.3\% \pm 26.1\%$) and intermittent ($103\% \pm 35.3\%$) application (9).

2. Equipment and application

Before initiation of therapy with vacuum assisted closure therapy basic principles of wound care have to be followed. The wound has to be properly debrided, to prevent any nidus for potential growth of bacteria. Incomplete debridement may lead to proliferation of granulation tissue over necrotic tissue, which might interfere with healing process, delaying healing and promoting abscess formation.

The wound is completely covered with foam dressing while special care has to be taken to cover the surface of the wound, including deep margins. The foam dressing is covered with an adhesive layer, which ensures a moist and warm healing environment. Connecting with the foam is the drainage tube, removing any excess fluid into the collection reservoir. The drainage tube is attached to the vacuum pump, which is able to create a pressure between -50 to -200 mmHg. Most commonly a negative pressure of -125 mmHg is used, except in some cases where less pressure is needed, for example in prevention of seroma in incision wounds for closed postoperative drainage, where a pressure of -50 mmHg is usually used (10)(11).

The operation mode of the pump can be either continuous, intermittent or variable mode of suction. Patients seem to tolerate continuous suction better, as it causes less pain (1). Comparing with continuous suction, intermittent suction however showed more granulation tissue formation and wound contraction (12).

The dressing is changed every 24 hours in the beginning of the treatment or more often if there is an active infection, and later approximately every 48 to 72 hours. The duration of the therapy depends on the type of wound and additional comorbidities and habits, such as smoking and diabetes. It usually lasts from days to weeks.

3. Indications

Vacuum assisted closure therapy is used with large, open and contaminated wounds, abdominal and thoracic wounds, chronic nonhealing wounds, skin avulsions and degloving injuries, as bolster for skin grafts, prevention of postoperative seroma and edema, as well as for the treatment of surgical dehiscence, myofascial compartment syndrome and burns (10)(14).

Vacuum assisted closure therapy is also increasingly used under appropriate supervision in home settings and primary health care, decreasing financial and organisational burden of hospital based care (15).

Table 1: Indications and guidelines for vacuum assisted closure therapy, following initial 48 hours continuous period.

According to Argenta (1997) (1)

| Type of wound | Cycle setting and subatmospheric pressure | Dressing change interval |
|---|--|-----------------------------------|
| Chronic ulcer (diabetic, dysvascular) | Continuous, 50 to 75 mmHg | 48 h (12 h with active infection) |
| Pressure ulcer | Intermittent, 125 to 175 mmHg | 48 h (12 h with active infection) |
| Acute, sub-acute, traumatic and dehisced wounds | Intermittent, 125 to 175 mmHg | 48 h (12 h with active infection) |
| Meshed graft | Continuous, 75 to 125 mmHg | Remove dressing after 3 to 5 days |
| Fresh flap | Continuous, 125 mmHg | 72 h (12 h with active infection) |
| Compromised flap | Continuous, 125 mmHg | 48 h (12 h with active infection) |

4. Risks, complications and contraindications for vacuum assisted closure therapy

Potential severe complications of vacuum assisted closure therapy are pain, bleeding, wound infection and communication between the intestine and skin (enteric fistula) (5).

Bleeding can occur as a consequence of an incomplete hemostasis during the wound preparation or overlooked fragile or open blood vessels.

Wound infection, which can lead to sepsis, can be caused by incomplete debridement of the wound as well as inability to visually check the healing of the wound and to drain pus collection on time (14).

VAC-treated wounds, such as pressure sores, infected wounds and skin grafts are painful by themselves, and pain can also be exacerbated by dressing changes. With therapy initiation, patients can sometimes feel stretching and pulling around the wound (16). Fewer dressing changes in combination with strategies to minimize pain are used. Only a small percentage of patients reported vacuum assisted closure treatment as painful in a small study conducted, while none of the patients withdrew from the therapy (17).

Possible contraindications for the usage of vacuum assisted closure are (1)(18):

- Malignancy
- Untreated osteomyelitis within the wound
- Non-enteric and unexplored fistula
- Necrotic tissue with eschar present

Precaution should be taken with (1):

- Active bleeding, difficult wound haemostasis and anticoagulant therapy
- Close proximity to blood vessels/ organs
- Irradiated or sutured blood vessels or organs
- Allergy to adhesive dressings
- Fragile skin, such as from aging or long-term use of topical steroids

5. Inflammatory response

Inflammation enhances the quality of tissue repair. Acute inflammation is an immediate response to the tissue damage and is promoted by chemoattractants derived from plasma proteins, resident and recruited hematopoietic cells, extracellular matrix and bacteria.

Efficient healing is mediated by polymorphonuclear cells, monocytes/ macrophages, mast cells and T-cells (13).

5.1. *Neutrophils*

After tissue injury, neutrophils and platelets, trapped in the blood clot, release factors, initiate the coagulation cascade and chemoattract cells involved in the inflammatory phase. In the next few hours most of neutrophils migrate through the endothelium of capillaries. Activation with the proinflammatory cytokines interleukin- 1β , tumour necrosis factor- α , and interferon- γ leads to the expression of adhesion molecules, such as P and E selectin and intercellular adhesion molecule 1 and 2. An interaction between adhesion molecules and integrins on neutrophils is needed for diapedesis and leucocyte adhesion.

Neutrophil recruitment depends on the interleukin-8, monocyte chemoattractant protein-1, growth-related oncogene- α , as well as bacterial products, such as lipopolysaccharides and formyl-methionyl peptides.

During this process antimicrobial substances such as high levels of bactericidal reactive chemical species under the catalysation of NADPH oxidase, myeloperoxidase, or nitric oxide synthetase (19) and proteases are released, needed for removal of devitalized tissue and phagocytosis of infectious agents (13).

5.2. *Monocytes/ macrophages*

Major portion of macrophages is recruited from the blood within two days after injury. Their role is phagocytosis of neutrophils, after the neutrophil infiltration ceases.

Besides resident macrophages, already present at the site of the wound, the infiltration is highly regulated by chemotactic factors, such as proinflammatory cytokines and growth factors, as well as chemokines. Major sources are platelets, keratinocytes, fibroblasts and leucocytes. With a help of Toll-like receptors, complement receptors and Fc receptors in the microenvironment of the wound, monocytes differentiate and mature into the tissue macrophages (13).

5.3. *Mast cells*

Mast cells are a subset of leucocytes, present in the skin and are involved in the tissue repair. Resident mast cells degranulate within hours after injury and the level returns to normal within 48h post-injury. As the tissue repair proceeds, the number of mast cells increases. Recent studies show significant impact of mast cells deficiency on influx of polymorphonuclear cells, vascular permeability and consequently on the wound closure rate (13).

5.4. *T cells*

T cells constitute the most prevalent subset of leucocytes during the phase of tissue remodelling and after the completion of the wound closure. Chemokines interferon- γ -inducible protein-10 and monokine induced by interferon- γ are crucial mediators for lymphocyte chemotaxis and function. The main source of these cytokines seem to be macrophages. Th1 and Th2 subsets differentially regulate the wound microenvironment by releasing different cytokine profiles. Th1 predominantly secrete interferon- γ , interleukin-2, and tumour necrosis factor- α while Th2 release interleukin-4, interleukin-5, and interleukin - 10. In addition, T cells directly influence cell to cell interaction via membrane bound glycoprotein CD40 and CD40 ligand.

Additionally, skin $\gamma\delta$ T cells, designated epidermal dendritic cells, recognize transformed keratinocytes in the epidermis. T cells are important source of key growth factors such as fibroblast growth factor-7, fibroblast growth factor-10 and insulin-like growth factor-1, important for keratinocyte proliferation and differentiation. It was also shown to influence keratinocyte-mediated hyaluron deposition in the extracellular matrix and acting on macrophage infiltration in the wound (13)(20).

6. Chronic wounds

Vacuum assisted closure therapy is predominantly used for the treatment of complicated and chronic wounds. Chronic wounds do not follow normal pattern of repair but are instead in a state of chronic inflammation. Three factors crucial for the lack of healing are local ischemia, infection and tissue swelling, which strongly inhibit wound regeneration (21). In addition, growth and survival of microorganisms in the infected wound depend on biofilm formation. A biofilm is a bacterial matrix which is supplemented by polymers (polysaccharides, proteins) and nucleic acids. Local microenvironment in the biofilm enhances bacterial colony formation, as well as ensures bacterial protection and adhesion. Biofilms are, in addition, providing adequate local wound moisture and facilitate signal transmission which transfers information responsible for drug resistance.

After surgical removal, biofilms constantly regenerate, with reconstitution within two to three days following primary removal. Resorption is therefore crucial for the process of wound healing.

Biofilms represent a barrier which decreases diffusion of respiratory gases and neutralizes penetration of antiseptics or anti-inflammatory solutions. Electrochemical gradient is supported by keeping pH relatively stable and structure supports growth of aerobic and anaerobic bacteria (22).

Due to an abundance of polymorphonuclear cells and macrophages, there is an increase in proinflammatory cytokines, as well as proteolytic activity and pro-oxidant microenvironment. This leads to direct damage by reactive oxygen species and to the degradation of growth factors and structural proteins of the extracellular matrix. Prolonged and unsuccessful healing is contributed by bacterial components such as extracellular adherence protein, formyl methionyl peptides and *N*-acetylmuramyl-L-alanyl-D-isoglutamine (13).

In addition, the importance of treatment of chronic wounds is in a possible malignant transformation. It can occur either due to the tumour stroma directly stimulating malignant transformation or due to environment which is rich in nutrients, because of highly vascularised and growth factor rich tumour stroma.

7. Influence of negative pressure on the bacterial growth and biofilm production *in vitro*

In order to interrupt wound healing there have to be $>10^5$ colonies of bacteria per gram of tissue (23). Negative pressure wound therapy affects bacterial colonisation by accelerating blood flow, increasing oxygen tension and enhancing neutrophil function, consequently resisting the infection by oxidative burst mechanism (24).

Studies suggest that vacuum assisted closure therapy effectively helps to eliminate biofilms and inhibits new biofilm formation. The proposed mechanism is through decompression of tissue, by separating discharge and reducing residual edema, resulting in significantly improved blood perfusion and lymphatic drainage (21).

Bacterial removal is the result of mechanical elimination of bacterial cells, as well as improved pharmacodynamic and pharmacokinetic features of drug penetration.

To test the effect of negative pressure on the bacteria and biofilm development *in vitro*, the researchers cultivated *Staphylococcus aureus* strain with constitutive expression of the green fluorescent protein. It was chosen because of its ability to be cultivated *in vitro*. It is also a predominant pathogen in hospital- and community-acquired wound infections (25).

Methicillin resistant *S. aureus* is often implicated in chronic wound infections (26).

The strain was grown overnight and subcultured in Luria-Bertain broth at 37 degrees Celsius, until reaching value equivalent to 10^6 colony forming units. Bacterial colonies were treated in the incubator with the negative pressure of -125 mmHg. Oxygen tension was kept constant by introducing adequate amount of room air in the incubator every 15 minutes. The control was treated in the ambient pressure under otherwise the same conditions.

Macromorphological characterisation was carried out by two independent observers blinded to treatment, observing shape, colour, size and surface. In addition, the presence of some of the exotoxins secreted by *S. Aureus*, was analysed by collecting an equal volume (25 microliters) of supernatant from cultures with negative pressure and the control. Western

blot analysis was performed to determine relative amount of α -hemolysin and extracellular adherence protein, expressed as the ratio of its mean optical density to the control.

Biofilm formation was measured by staining the static biofilm assays with 1% crystal violet, rinsed with phosphate buffered saline and observed under the microscope. To quantify total biofilm formation, absorbed stain was washed with 95% ethanol and absorbance was read with spectrometer (wavelength = 595 nm). In addition, Concanavalin A staining for biofilm matrix was used, which detects exopolysaccharides and is seen with fluorescence microscope.

Another method for measuring the presence of biofilms is to measure the presence of polysaccharide intercellular adhesin, extracellular DNA and proteins. Static biofilms were mixed with ethylenediaminetetraacetic acid (EDTA) and suspended in TES buffer (10 mM Tris HCl, 100 mM NaCl, and 1.0 mM EDTA at a pH of 7.8) (27).

Supernatant was used to measure the absorbance of the reaction solution at 585 nm read by a spectrometer. The concentration of eDNA was expressed as the concentration of isolated DNA from 100 microliters of the supernatant.

The results of the experiment showed visually changed macromorphological characteristics, including changed pigmentation in the culture under negative pressure, suggesting less carotenoid production. Colonies were smaller and the growth rate was significantly decreased in the group treated with negative pressure.

Negative pressure resulted in significant decrease in eDNA and PIA, suggesting inhibitory effect on biofilm formation. In addition, negative pressure caused decrease in the total production of α -hemolysin and EAP without significantly affecting protease secretion.

Microscopy showed fewer bacteria attached, suggesting reduced biofilm formation. Decrease in biofilm formation was proven as absorbed stain was eluted with ethanol and quantified spectrophotometrically at an absorbance of 595 nm showing significant decrease in biofilms formation comparing to control. Furthermore, intact biofilm structure with a large

amount of exopolysaccharide matrix around bacteria was detected in the control group. The negative pressure group lacked a biofilm matrix.

Quantitative real-time PCR was used to investigate the mechanism of negative pressure induction in attenuating biofilm production and inhibiting virulence factors of *S. aureus*. It was found that negative pressure inhibits the transcription of intracellular adhesion A gene, holing-like protein, α -hemolysin and extracellular adherence protein gene. Expression of those genes is regulated by the accessory gene regulator system and by main effector molecule RNAIII which were investigated and shown to be significantly repressed under negative pressure (28).

8. Influence of negative pressure on the bacterial growth and biofilm production *in vivo*

Despite proven benefits in *in vitro* studies, *in vivo* studies had to show similar results of the beneficial effect of the negative pressure, for the possible use in the clinical treatments.

Tongtong et al. (25) conducted a research in 2020, during which adaptive expression of biofilm regulators and adhesion factors of *Staphylococcus aureus* were observed. The research was done on the rabbit model of acute wound infection. A standardised 3 cm-diameter full-thickness dermal wound was created on the sterilized surgical site. Two wounds per rabbit were inoculated with a total of 1×10^7 CFUs of *S. aureus* at a volume of 0.1 ml.

Wounds were randomly and respectively assigned to untreated control side and negative pressure wound therapy. Additionally, rabbits were divided between group A, where bacterial count, gene expression analysis and healing conditions were observed (n=10) and group B where histological study was conducted (n=8).

Wounds treated with continuous negative pressure of -125 mmHg, were checked daily and the dressings were changed every 48h or when imaging was required.

On the contralateral side wound was bandaged, following otherwise the same protocol as the wound treated with the negative pressure and acted as the untreated control.

When histologically examined, untreated control group showed numerous discrete aggregated and microcolonies of bacteria, with local necrosis and cavitations. Wounds treated with negative pressure presented with healthier granulation bed without local necrosis.

Laser scanning confocal microscopy showed bacteria that appeared as clusters and biofilms in untreated control group. Conversely, bacteria in the wounds treated with negative pressure appeared on scanning electron microscopy as single cells or diplococci, lacking an extracellular matrix.

There was a significant decrease in viable bacteria level in negative pressure wound therapy group, however on the day 8, there was still a considerable number of bacteria in the tissue (1.16×10^6 CFUs/g).

Reverse transcription-quantitative PCR analysis was used for analysis of biofilm regulators of *Staphylococcus aureus* in response to negative pressure wound therapy. Levels were, similar as in previous experiment, presented as fold changes relative to the inoculum, which was used as a calibrator with a value of 1. Negative pressure treatment significantly decreased levels of poly-beta-1,6-N-acetyl-D-glucosamine synthase and holin-like protein CidA, increased antiholin-like protein LrgA and did not significantly affected LysR family regulator protein CidR levels. The presented data in conclusion showed decrease in biofilm formation.

Negative pressure treatment significantly upregulated the expression of adhesion factors associated with bacterial colonization, such as UDP-phosphate N-acetylglucosaminyl 1-phosphate transferase, fibronectin-binding protein A and iron-regulated surface determinant A levels compared with untreated wounds throughout the time course. The authors suggested that increased levels of adhesion factors correlated with decreased levels of bacteria in the local niche built by negative pressure and with commensalism of *S. aureus*. The spread of invasive infection is however regulated by agr quorum-sensing system, which is cell density dependent regulatory mechanism, with the main effector molecule RNAIII. RNAIII levels were downregulated relative to the control group, which shows that despite enhanced adhesion of bacteria, bacterial density in negative pressure treated wounds was under threshold for agr activation, which is the main factor that would lead to active and invasive infection. Lastly, on the day 24, wounds treated with negative pressure were completely healed, covered with neopithelium, while large skin defects were present in the control group. Negative pressure treatment therefore positively influenced the wound healing.

In a different clinical study in 2009 an interprofessional expert advisory panel reviewed infected wound treatment methods within evidence-based medicine together with VAC Therapy Systems to manage a variety of infected wounds (19).

The article was published in the International Wound Journal, as a clinical review and case series, where authors observed direct effect on the infected wounds in four case studies.

To establish an algorithm for treatment of infected wounds, wounds were divided in the four categories, depending on the level of colonization.

8.1. *Contaminated wounds*

Contaminated wounds are defined by the presence of non-replicating microorganisms, which do not impair wound healing. The goal of the treatment is sufficient debridement of the wound to prevent further wound deterioration.

8.2. *Colonized wounds*

Wounds that demonstrate granulation tissue, wound contraction and decreased wound depth with bioburden low enough to not impair the wound's ability to heal are classified as colonized wounds. As most chronic wounds contain multiple species of microorganisms, identifying causative organism can be difficult. Broad spectrum antibiotic in combination with debridement and negative pressure wound therapy is warranted.

8.3. *Critically colonized wounds*

The expression is used synonymously with locally infected wound. Level of bacterial burden is higher than in the category of colonised wounds, however it remains below the threshold of infected wounds. These wounds do not heal but as well do not display classic local signs of infection (erythema, warmth, pain, redness and swelling). Critically colonized wounds are

at very high risk of becoming infected, as they do not demonstrate normal host response to infection and are often contaminated with bacterial colonies producing biofilms.

8.4. *Infected wounds*

In contrast to critically colonized wounds where biofilm is present with early local infection of soft tissue, infected wounds have, additionally to soft tissue infection with biofilm, also a characteristic spread to the area around the wound.

Depending on the type, pathogenicity, as well as quantity of organisms, infected wounds may demonstrate any of the classic signs of infection.

It was established that negative pressure wound therapy can be used with critically colonised or infected wounds, however patients have to be free of most of the systemic signs of gross infection, such as fever, increased white blood cell count, CRP etc. In addition, wound has to be debrided of all necrotic tissue, abscesses have to be drained and there has to be adequate perfusion of the wound and surrounding area.

Four case studies involved patients between 11 and 70 years old, which had different difficult to manage wounds of the lower extremities.

Case 1 was an 11-year-old male with acute lower extremity soft tissue avulsion injury and tibia/fibula fracture. Large soft tissue deficit with heavy contamination of the wound was present. After debridement and external fixation foam with silver dressing was applied on the wound at the negative pressure of -125 mmHg. On day 7 patient returned to the operating room to receive split thickness skin graft and continue negative pressure therapy at the -75mmHg. On the day 3, after skin grafting, dressing was removed with 100% graft take.



Figure 4A: Acute lower extremity soft tissue avulsion injury on 11-year-old male.

According to: Gabriel (2009) (19); used with permission.



Figure 4B: Wound with VAC installation.

According to: Gabriel (2009) (19); used with permission.



Figure 4C: Split thickness skin graft after VAC therapy.

According to: Gabriel (2009) (19); used with permission.

Case 2 was a 49-year-old male with a chronic deep infection of the distal tibial fracture. After initial debridement and removal of the hardware, negative pressure therapy was initiated at -125mmHg, and later converted to foam with silver dressing

When returned in the operating room, bilaminar skin substitute and final coverage with split thickness skin grafts were performed with satisfactory results.



Figure 5A: Chronic deep infection of the distal tibial fracture on a 49-year-old male patient.

According to: Gabriel (2009) (19); used with permission.



Figure 5B: Preparation of the wound on VAC installation.

According to: Gabriel (2009) (19); used with permission.



Figure 5C: Result after VAC therapy, bilaminar skin substitute and final coverage with split thickness skin grafts.

According to: Gabriel (2009) (19); used with permission.

Case 3 was a 65-year-old patient with a three-month history of infected open right knee joint and exposed hardware. Wound was critically colonised and positive for the presence of biofilm.

After debridement the wound was continuously irrigated with silver nitrate to break down existing biofilms and prevent new biofilm formation. On day 10 of the negative pressure therapy improved healing enabled closure of the knee via local flap.



Figure 6A: Infection of a right knee joint with exposed hardware.

According to: Gabriel (2009) (19); used with permission.



Figure 6B: Wound with VAC installation.

According to: Gabriel (2009) (19); used with permission.



Figure 6C: Closure of the wound after VAC therapy and local flap

According to: Gabriel (2009) (19); used with permission.

The last case was a 70-year-old male who underwent above the knee femoral and popliteal artery bypass grafting for peripheral vascular disease and osteomyelitis of the left foot. Three months later he presented with cellulitis and fever of the medial distal thigh. Due to elevated white blood cell count and presence of abscess with MRSA on CT scan, patient was hospitalised and irrigation and debridement of the wound were done. Irrigation vacuum assisted closure therapy was used, which was switched to regular negative pressure wound therapy after day 9, when granulation tissue started to form.

At two-months follow up, the patient's wound was without infection and the wound remained closed.



Figure 7A: Primary wound of artery bypass grafting after debridement and irrigation.

According to: Gabriel (2009) (19); used with permission.



Figure 7B: Wound with VAC installation.

According to: Gabriel (2009) (19); used with permission.



Figure 7C: At 2-months follow-up the wound remained closed and without an infection.

According to: Gabriel (2009) (19); used with permission.

During the treatment different modalities of vacuum assisted closure therapy were used. Negative pressure wound therapy with or without instillation can be used (29). Irrigation is done with antiseptic solutions, for example 0.5% silver nitrate, combination of the electrolyzed water, sodium chloride, sodium hypochlorite and hypochlorous acid, or regular sterile water irrigation. Combination of the electrolyzed water, sodium chloride, sodium hypochlorite and hypochlorous acid protects against wound dehydration and contamination and help with wound exudate absorption (30). In addition, dressings with or without silver were used, depending on the type and infection of the wound. Silver has strong antimicrobial activity, which benefits wound healing in the first few days to weeks of healing (31).

9. Discussion

Vacuum assisted closure therapy has positive effects, proven both *in vivo* and *in vitro*, according to the collected data. The amount of research is in general sufficient to prove beneficial effect of negative pressure on management of difficult wounds.

There is lack of research done with gram negative bacteria, as well as with some rare bacterial species. Possibly the lack of research stems from the fact that some bacterial species are difficult to cultivate *in vitro*. *In vivo* chronic wounds often contain three or more bacterial species and therefore overall effect on the wound granulation, rate of closure and cardinal signs of inflammation are observed. In addition, presence of biofilms in the wounds is often tested and the effect of negative pressure on it is followed. More research could be done with biofilms, observing overall effect on gene regulation, not just on few secluded gene sequences, which were observed in most of the studies.

Additionally, when comparing different articles standardisation is difficult as research was conducted under different operation modes of vacuum assisted closure systems, produced by different companies. In addition, there are different types of foam used. White foam is used with superficial and less severe wounds with minimal exudate, as well as a protection over split thickness skin graft. In more inflamed wounds silver is often added to the covering foam.

The limitation of the method lies in limited usage with circumferential wounds as well as wounds on distal extremities on patients with comorbidities such as peripheral artery disease.

10. Conclusion

Negative pressure wound therapy is a method which has a proven usefulness and efficiency as an independent therapy of managing difficult wounds as well as an adjunctive therapy with surgery and antibiotic treatment. It has proven its significance with wounds that contain biofilms, as well as those heavily contaminated with multiresistant bacteria. The vacuum assisted closure therapy is able to physically break biofilms and contributes to increased cleaning of the wound, better closure and granulation tissue rate, and consequently to increased sensitivity to the antibiotics.

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Biography

Viviana Wahl was born in Ljubljana, Slovenia, on 15th of September 1996. After finishing elementary school, she started high school in Kamnik for two years, before starting IB program in Ljubljana. After high school she followed her dreams to become a medical doctor and study in English, so she started studying on Medical faculty at University of Zagreb.